IDENTIFICATION OF RISK FACTORS ASSOCIATED WITH FOOD WASTE REDUCTION

A Thesis

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by

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ABSTRACT

Food waste occurs from initial production all the way to consumption. Whilst different tactics are implemented to reduce food waste among the industry and consumers, changes in production and distribution methods change the sources and degree of risk. Understanding and identifying risk factors that might be introduced through changes, such as product handling and pattern of use, are needed to ensure food safety. The use of outgraded produce (i.e., visually unacceptable for the market) and the proposal of ignoring shelf life date labels have gained attention as solutions to food waste in both the U.S. and Europe. This study suggests that outgraded produce with physical damage (i.e., open lesions) retains more pathogens following disinfection treatment compared to ones with physiological defects (i.e., wounds with intact surface). However, rapid growth of spoilage microbiota limits the shelf life of outgraded produce with physical damage, and thus makes the survival and growth of retained pathogens, during post-harvest storage, irrelevant to food safety. In contrast to whole fruits and vegetables, the growth of *L. monocytogenes* in physically damaged produce became problematic before consumers could detect the sensory deterioration on RTE foods under both strict and abuse refrigeration temperatures. Therefore, the quality deteriorations, such as off odor, sliminess and fungal growth, should not be used as fail-safe indicators considering shelf-life limitation for *L. monocytogenes* growth. This study addresses food safety concerns associated with waste reduction and provides a quantitative framework for the development of risk management decisions.

BIOGRAPHICAL SKETCH

Shiyu Cai was born and raised in Shanghai, China and came to the United States to pursue higher education in 2012. She attended Purdue University in West Lafayette, Indiana and acquired a Bachelor of Science degree in Food Science in 2016. During her time at Purdue University, Shiyu developed a keen interest in food microbiology and food safety. She is currently in her second year of a master's study in Food Microbiology at Cornell University. In December 2017, Shiyu will graduate with a Master of Science degree, with a focus in Food Microbiology. She has completed numerous food safety and quality assurance courses, and has earned many certifications including the Food Safety Modernization Act (FSMA) Preventive Controls Qualified Individual, Implementing SQF Systems (Post-Farm Gate), Good Manufacturing Practices (GMP), and Hazard Analysis Critical Control Practices (HACCP).

In the past one and a half years, Shiyu has served as a teaching assistant and an extension assistantship in the Sensory Evaluation Center at Cornell University. These experiences provided her with leadership skills and valuable knowledge-learning experience outside of her field. She has also participated in teaching a Juice HACCP Certification Course and enjoyed training individuals from the industry on developing HACCP plans and reviewing records. In an international student innovation contest held by FiberStar Inc., Shiyu, along with six team members, placed sixth with honors. In her spare time, Shiyu enjoys rock climbing and reading to balance her life outside of Food Science.

In the short term, Shiyu looks forward to beginning her Ph.D. program at the Ohio State University on food spoilage prevention associated with industrial food handling practices.

To my mom Jiahong Ma, my dad Yong Cai, and my boyfriend Hans Duong

I dedicate this thesis

谨以此献给我的母亲，父亲和我的男友

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LIST OF ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Unit
CVTA	Crystal Violet Tetrazolium Agar
FDA	US Food and Drug Administration
FSIS	USDA's Food Safety and Inspection Service
LMPM	*Listeria monocytogenes* Chromogenic Plating Medium
LOD	Limit of detection
MA	Modified atmosphere
MAP	Modified atmosphere packaging
MRS	de Man, Rogosa and Sharpe Agar
OTR	Oxygen transmission rate
PDA	Potato Dextrose Agar
rif	rifampicin
RTE	Ready-to-eat
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
USDA	United States Department of Agriculture
WHO	World Health Organization

CHAPTER 1
OUTGRADED PRODUCE VARIABLY RETAINS SURFACE INOCULATED *ESCHERICHIA COLI* THROUGH DISINFECTION TREATMENT

1.1 Abstract

The use of outgraded produce that does not meet supermarkets' cosmetic standards has gained attention as a solution to food waste in both the U.S. and Europe. The purpose of this study was to evaluate the impact of using outgraded produce on the retention of surface inoculated *E. coli* following disinfection treatment on four model fresh produce systems (apple, tomato, carrot, lettuce). A three-strain cocktail of rifampicin-resistant generic *E. coli*, with a concentration of 9.0 log CFU/ml, was spot-inoculated on the intact surfaces of U.S. No.1 grade produce items and damaged or decayed areas of outgraded produce items. Generally, outgraded produce of all four kinds retained significantly higher levels of inoculated *E. coli* following two postharvest treatments, chlorinated (150 ppm) and water only. Treating with 150-ppm chlorine, normally known as chlorine dip, was not sufficient to eliminate the additional risks from using secondary quality or outgraded produce, and the efficacy of disinfection was greatly affected by type of deviation. Produce with bruises or broken skins were labeled as having "Physical Damage", while the rest were said to have "Physiological Defects". Although chlorine dip represents only a modest reduction in pathogens generally, the results from this study suggest that outgraded produce with physiological defects may pose less food safety risks if introduced into the fresh market than does that with physical damage due to their enhanced retention of bacterial cells. Therefore, as industry considers how to minimize its food waste problem, preferentially directing physically damaged produce away from the fresh market will help to minimize risk while maximizing food resources.

1.2 Introduction

According to the publication of the Food and Agriculture Organization of the United Nations (2011) on global food losses and food waste, roughly 45% of the fruits

and vegetables (including roots and tubers) produced for human consumption are discarded, lost, or uneaten. Compared to all other food groups (meats, dairy, etc.) fruits and vegetables have the highest wastage rate. In the U.S., the USDA Agricultural Market Service grades produce based on certain quality characteristics and certain markets require certain grades of produce. The U.S. No. 1 standard requires produce to be at least fairly smooth on the surface, fairly well colored, fairly well formed, and free from decay (see Table A1.1 in Appendix for full descriptions). Fruits and vegetables that do not meet the U.S. No. 1 standard are often outgraded from commercial sale. Out-grading of blemished, misshapen, or wrong-sized foods due to minimum quality standards set by the federal marketing orders and consumer's expectation of cosmetic perfection lead to non-harvest and culling of edible produce (Powers, 1990). Even though outgraded fruits and vegetables are sometimes used for processing, most large processors in the United States themselves have product specifications (e.g. varieties specific for processing) which limit this waste recovery strategy (NRDC, 2012).

Many countries have recognized food waste as an important issue threatening food security. In the U.S., 12.7% of U.S. households were reported as food insecure at some time throughout the year, meaning these households lacked access to enough food for all household members due to insufficient resources (Coleman-Jensen et. al, 2016). In the U.K., the emergency food aid provided to impoverished households increased by 163% over the course of one year from 2013 to 2014 (Loopstra et al., 2015). Food losses between farms and retailers cause lost income for farmers and higher prices for consumers. Under such circumstances, secondary quality fresh produce has gained increased attention as a solution to food insecurity in both the U.S. and Europe. Retailers are gradually bringing blemished fruits and vegetables into the market. With the increasing sales and consumption of deformed and blemished fresh fruits and vegetables, there is a need to evaluate potential risks to food safety that may be introduced. This is especially relevant when considering that food insecure consumers are often more vulnerable to disease sequela associated with foodborne disease.

Fruits and vegetables consumed raw pose a food safety risk since no kill step is typically applied to fresh produce. The CDC reported that from 2002-2011, 667 outbreaks (17% of total) were associated with produce category and 23,748 people (24% of total cases) in the United States were sickened from consuming contaminated fresh produce. The number of produce-associated outbreaks exceeded all other food types and caused, on average, the largest number of illnesses per outbreak (DeWaal & Glassman, 2014). Many produce commodities are susceptible to contamination from soil, irrigation water, wild and domestic animals, and inadequately composted manure prior to harvest (Cooley et al., 2007; Jay et al., 2007). U.S. produce packers and the fresh-cut industry commonly use disinfection treatment following a triple-wash technology with low concentrations of chlorine, peracetic acid, or other sanitizing agents to reduce the incidence of cross-contamination and improve the safety of their products. The disinfection treatment is known to remove around 1-2 log CFU/g of microbial pathogens (Parish et al., 2003; Akbas & Olmez, 2007; Lee et al., 2014; Snyder et al., 2016).

However, the efficacy of sanitizer on bacterial inactivation depends on types of produce and contamination sites (Alvarado-Casillas et al., 2007; Olaimat & Holley, 2012; Snyder et al., 2016). Plant development is affected by growing conditions and unfavorable weather, which induce undesirable defects. Many researchers have found that growth cracks, porous and broken tissue, and wounds provide a protective environment for pathogens against disinfection treatment on various types of fruits/vegetables (e.g. Burnett et al., 2000; Takeuchi & Frank, 2000; Han et al., 2001; Stopforth et al., 2004; Wang et al., 2009). In addition, internalization occurs during post-harvest cooling and washing steps, where water may be a vehicle for pathogen internalization through deformed sites, and temperature differentials cause surface-borne microbes to ingress through wounds, lesions, and growth cracks. Studies have shown that once pathogen cells penetrate plant tissues deeper than 4.2 mm, sanitizer treatments are virtually ineffective (Fatemi & Knabel, 2006). The degree to which these defects protect bacterial contaminants varies by the type and severity of damage. Wei et al. (1995) suggested the effectiveness of such protection depends on the cause

of the damage (e.g. physical wounding, pest damage, or plant disease). Shallow cracking to the fruit cuticle may not harbor microbes to the same extent as those that extend through to the interior tissue of the fruit. Additionally, the exposure of plant nutrients may facilitate growth of pathogens, while the application of antimicrobials may have decreased efficacy.

In order to reduce fresh produce waste and increase access among food insecure populations, risk identification and proper risk management strategies are needed to protect food safety. This study evaluated the efficacy of disinfection treatment to decontaminate secondary quality or outgraded produce with surface inoculated *E. coli* on four model fresh produce systems.

1.3 Materials & methods

1.3.1 Bacterial strains & inocula preparation

A cocktail of rifampicin resistant derivatives of generic *E. coli* strains was used to inoculate produce. The cocktail contained TVS 353 (derived from *E. coli* W778), TVS 354 (derived from *E. coli* P149) and TVS 355 (derived from *E. coli* S19) as described by Tomas-Callejas (2011). These strains were originally isolated from surface irrigation water, Romaine lettuce, and sandy-loam soil samples (Salinas Region, CA, USA), respectively, and have been utilized as model pathogens in fresh produce production systems (Tomas-Callejas et al., 2011). Bacterial cultures were stored at -80°C in Tryptic Soy Broth (TSB) (Becton, Dickinson and Co., Sparks, MD) containing 25% (vol/vol) glycerol. To prepare the inocula, the three generic *E. coli* strains were grown separately in 9 mL TSB supplemented with 100 mg/L of rifampicin (EMD Millipore Corp., Billerica, MA), and incubated at 37°C for 18 h on a rotary shaker (200 RPM). After incubation, *E. coli* cells were harvested by centrifugation (13,000 RPM, 10 minutes), re-suspended after being washed twice in phosphate-buffered saline (PBS, pH 7.0, Fisher Chemical, Inc., Fair Lawn, NJ). The three bacterial suspensions were combined and the final concentration of the inocula was determined by plating on Tryptic Soy Agar supplemented with 100 mg/L of rifampicin (TSA-rif) to be about 9.0 log CFU/ml.

1.3.2 Fresh produce selection & grading

Four types of fresh produce, tomatoes (BHN 589, Cornell University, Geneva, NY), apples (Cortland, Gala, Honeycrisp, Cornell University, Ithaca, NY), carrots (Imperator, purchased from a specialty grower-seller) and lettuce (Romaine, purchased from a commercial retailer), were selected to represent a wide variety of common fruits and vegetables in the market. For tomatoes, apples, and carrots, 100 items containing 50 U.S. No.1 quality items and 50 secondary culls were obtained. For Romaine lettuce, ten whole heads of lettuce were obtained from the same lot at a commercial retailer (Ithaca, NY). Produce items were collected throughout October and November 2016. All the selected produce items were held at 4°C for up to 48 h until use.

Tomatoes, apples, carrots, and lettuce were graded categorically as "U.S. No.1", "Injury", "Damage" and "Serious Damage" according to the USDA Market Inspection Instructions (USDA, 2004 & USDA 2005) by trained researchers, and the assignment of degree and type of defect was verified by an independent fruit/vegetable physiologist. For each experimental condition, 25 U.S. No.1 and 25 outgraded (Injury, Damage, Serious damage) produce items were selected, but the numbers of each grade were not controlled. Type of defects for tomatoes included growth cracks, catfaces, zippers, bruises, insect stings, and shapes (USDA, 2005). Defects on apples were mainly russeting, insect stings, lesions, scald and shapes (USDA, 2005). Russeting was caused by apple rust mites feeding on fruitlets, whereas insect stings were identified as damages caused after apple maturity. Forking or deformity with a few cracks/holes was observed in secondary quality carrots (Fig. 1.1). For lettuce, the outgraded quality leaves were taken from the exterior of the lettuce head and were characterized by blemishes and wilt, and are frequently removed by retailers before sale (Fig. 1.1). These defects were identified as physical damage and pink ribs (USDA, 2004). Meanwhile, the internal lettuce leaves without any damage was considered the highest quality produce. Because selected produce was harvested throughout October and November, other types of defects that emerge seasonally may

not have been captured in this study. In addition to the quality grade, the type of defect and the weight of the produce were recorded. Cohen's kappa coefficient was calculated to determine the inter-rater agreement and was determined to be 0.92.

Defects on outgraded apples and tomatoes were further categorized into "Physiological Defects" and "Physical Damage" (Table A.1). Produce with physiological defects had no bruises or broken skins, and vice versa. Defects, such as russeting, scald, shapes from outgraded apples, zipper and shapes from outgraded tomatoes, were viewed as physiological defects. Physical damage included insect stings, lesion, bruises, catfaces, growth cracks, broken skins and cuts.

1.3.3 Inoculation of fruits and vegetables

Fresh produce was spot-inoculated with 10 ×10-μL aliquots of the *E. coli* cocktail inocula. Secondary quality produce was inoculated on the sites of damage or defect. Intact surfaces were inoculated on U.S. No.1 quality produce. A 2-inch×2-inch square was marked with permanent markers for later identification of all inoculation sites. Inocula were allowed to air dry for 2 h, at ambient temperature, prior to treatment.

1.3.4 Treatments

A 1.5 L volume of deionized water with agitating in a 5 L beaker was used in the treatment of tomatoes, apples, and lettuce at room temperature. Aqueous chlorine (150 ppm) was added as disinfection treatment condition for half the produce. Inoculated produce was completely submerged in the tank and treated for 1 min in aqueous chlorine followed by a 1 min rinse in water alone. Produce treated without chlorine was washed for 2 min in water as control.

Dipping solution was changed after every six produce items, and residual free chlorine concentration was measured using free chlorine strips (2008 Industrial Test System, Inc., Rock Hill, SC) to ensure treatment conditions were maintained as stated throughout each run. Treated items were allowed to completely air dry for 1 h. Due to the different post-harvesting condition of carrots, farm-harvested carrots were treated

in 2 L deionized water for 10 min with agitation, followed by washing in a simulated drum washer with 2 L volume of potable water for another 15 min. Drum washing was simulated with 60 RPM shaking in order to remove soil from the carrots. For chlorine dip conditions, 150 ppm was added to the drum-washing step for 25 U.S. No.1 quality and 25 outgraded carrots.

1.3.5 Recovery and enumeration of bacteria

The inoculated sections were excised with sterile knives and transferred directly into sterile stomacher bags (Whirl-Pak, Nasco, Jackson, WI). Samples were 10-fold diluted with PBS based on weight and homogenized in a stomacher (STOMACHER® 400 CIRCULATOR) for 2 min at 230 RPM. Filtered homogenates were serially diluted and spread-plated on TSA plates containing 100 mg/L rifampicin. Plates were incubated at 37°C for 36 h and enumerated. Eight colonies per trial were verified by PCR amplification of the clpX gene (Walk et al., 2009). For U.S. No.1 quality tomatoes and apples that retained *E. coli* counts below the limit of detection, a subsample of the produce was evaluated to increase the sensitivity of the enumeration procedure. Ten U.S. No.1 quality apples and tomatoes were 5-fold diluted with PBS and 1 ml of homogenates spread-plated across three TSA plates containing rifampicin.

1.3.6 Statistical analysis

All statistical analyses were performed in R (version 3.3.1, R studio, Boston, MA). The Student's t-test was performed to evaluate statistically significant differences between the mean weight values of U.S. No. 1 and outgraded produce. For microbial count data, considering the large numbers of samples with counts under the limit of detection (Table A.2), a binomial logistic regression comparing detection (yes or no) of inoculated *E. coli* under different experimental conditions was performed using a generalized binary model with binomial family (Zeileis, 2009). Separate models were used for each produce type. Conditions in which majority of the 25 observations were below the limit of detection were excluded and the rest were instead analyzed in a subsequent linear regression based on quantitative microbial counts.

Pairwise comparison between treatments and quality grades were evaluated using least square means (lsmeans) package (Lenth, 2016). The Student's t-test was used again to evaluate statistically significant differences between *E. coli* count reduction following water only and chlorine treatment over each grade and type of damage.

1.4 Results

1.4.1 Weight of U.S. No.1 and outgraded produce items

No significant weight difference was observed between U.S. No.1 quality apples and outgraded apples ($p = 0.2791$). Outgraded tomatoes ($p = 0.0049$) and carrots ($p < 0.0001$) weighed significantly more on average than the U.S. No.1 quality counterparts. Outgraded carrots had a tendency of higher weight because the items were mostly carrots with roots intertwined or forked (Fig. 1.1). Outgraded tomatoes were often characterized by cuticle cracking or growth cracks on larger items, which may account for this difference (Fig. 1.1). As mentioned previously, the outgraded lettuce leaves were blemished and wilted outer leaves, however, the weight of outgraded lettuce leaves was not significantly different from the U.S. No.1 grade lettuce leaves ($p = 0.1044$). By excising the inoculated portion of the fresh produce, weight differences were normalized during *E. coli* enumeration.

Fig. 1.1 U.S. No.1 grade produce vs. outgraded produce.

1.4.2 Retention of generic *E. coli* following disinfection treatment

The counts of rifampicin resistant *E. coli* retained on treated U.S. No.1 grade

and outgraded fresh produce were measured and compared. A binomial logistic regression comparing detection (yes or no) of inoculated *E. coli* under different experimental conditions was performed due to the large numbers of samples with counts under the limit of detection (Table A2.2). Simply using 2 log CFU/g to replace the counts that were below limit of detection (< 2.0 log CFU/g) would result in inaccurate significances from the linear regression model. The binomial logistic regression was used to remove the experiment conditions with a high probability of counts under the limit of detection, so that the remaining data could be used for statistical analysis in linear regression model based on quantitative microbial counts. The p-value from the logistic regression showed that significantly more U.S. No.1 quality apples than outgraded apples under both chlorine and water treatments ($p < 0.0001$) were decontaminated to levels where *E. coli* counts were below the limit of detection (<2.0 log CFU/g). The *E. coli* counts retained on 24 out of the 25 U.S. No.1 quality tomatoes under chlorine treatment were below the limit of detection. However, the p-value from the logistic regression suggested that the number of observations wherein *E. coli* counts on U.S. No.1 quality tomatoes were below the limit of detection was not significant ($p = 0.9892$). This is because the water-treated U.S. No.1 tomatoes retained *E. coli* counts variably above and below the limit of detection, leading to the high p value for U.S No.1 quality tomatoes as a group. Similarly, the *E. coli* counts on 44% and 52% of the U.S. No.1 quality carrots were below the limit of detection under water only and chlorine treatment, respectively. Even though the p-value from the logistic regression suggested that the number of observations wherein *E. coli* counts on U.S. No.1 quality carrots were below the limit of detection was significant ($p = 0.0020$), the probability of *E. coli* counts under the limit of detection was not high enough when separating the observations under the two treatments. All water-treated and over half of the chlorine-treated U.S. No.1 grade lettuce leaves retained *E. coli* counts that were significantly above the limit of detection ($p = 0.9903$). Therefore, all the observations of U.S. No.1 carrots and lettuce leaves were kept for the following analysis.

Treatments resulting in detectable *E. coli* counts were subsequently compared

using linear regression (Fig. 1.2). Outgraded produce of all four kinds retained a significantly higher load of inoculated *E. coli* following disinfection treatment ($p < 0.0001$). Compared to U.S. No.1 quality apples, outgraded apples retained 4.3±1.4 log CFU/g more *E. coli* following water only treatment and 3.6±1.7 log CFU/g more following chlorine treatment. Outgraded tomatoes significantly retained 3.5±1.1 log CFU/g more inoculated *E. coli* following water only treatment and 3.0±1.4 log CFU/g more inoculated *E. coli* following chlorine treatment than U.S. No.1 quality tomatoes did under the same treatment conditions ($p=0.0087$). Outgraded carrots retained 1±1.1 log more inoculated *E. coli* following water only treatment and 0.5±0.8 log CFU/g more inoculated *E. coli* on following chlorine treatment, compared to U.S. No.1 carrots. Outgraded lettuce leaves retained 1.6±0.5 log CFU/g more inoculated *E. coli* following water only treatment and 4.1±0.4 log CFU/g more inoculated *E. coli* following chlorine treatment than did U.S. No.1 quality lettuce leaves under the same treatment conditions. Additionally, compared to water-treated U.S. No.1 grade produce, chlorine-treated outgraded produce had a higher bacterial retention. Chlorine-treated outgraded apples retained 3.0 log CFU/g more *E. coli* counts, than did water-treated U.S. No.1 grade apples ($p < 0.0001$). Chlorine-treated tomatoes retained significantly (4.0 log CFU/g) more pathogens than the water-treated U. S. No. 1 quality tomatoes ($p < 0.0001$). Using outgraded produce could potentially introduce additional food safety risks upon contemporary industry practices associated with fresh produce consumption. Meanwhile, application of chlorine treatment was not sufficient to eliminate the additional risks from using secondary quality or outgraded produce.

Chlorine treatment efficacy varied based on produce grade and type. Water only treatment was sufficient to reduce *E. coli* counts below the limit of detection on U.S. No.1 apples, and therefore, no significant difference was observed between water only treatment and chlorine treatment on U.S. No.1 quality apples. To increase the sensitivity of the enumeration procedure, a subsample of ten U.S. No.1 quality apples was evaluated by spread-plating across three TSA plates containing rifampicin with 1 ml of 5-fold diluted homogenates, and the subsample, on average, retained 0.83±0.61

log CFU/g *E. coli* counts following water only treatment and 0.06±0.12 log CFU/g *E. coli* counts following chlorine treatment. It suggested that the chlorine treatment gave a better microbial reduction on U.S. No.1 quality apples than did the water only treatment. Treating outgraded apples with chlorine resulted in a significant 1.4 log CFU/g reduction of *E. coli* ($p = 0.001$). Chlorine treatment significantly reduced *E. coli* counts on U.S. No.1 quality tomatoes, compared to the water only treatment. Water-treated U.S. No.1 quality tomatoes retained 2.86 log CFU/g, whereas the *E. coli* counts retained on U.S. No.1 quality tomatoes were below the limit of detection following chlorine treatment. The subsample of U.S. No.1 tomatoes, on average, retained 2.13±0.35 log CFU/g *E. coli* counts following water only treatment and 0.37±0.46 log CFU/g *E. coli* counts following chlorine treatment. Outgraded tomatoes retained significantly 0.94 log CFU/g more inoculated *E. coli* counts than did those following chlorine treatment ($p = 0.0133$). Results above showed that chlorine treatment significantly reduced surface-inoculated *E. coli* counts retained on outgraded apples and tomatoes. This is because the physiological defect with intact cuticles on outgraded apples and tomatoes prevented microbes from deep internalization. However, chlorine treatment was not significantly more effective in microbial reduction on carrots or outgraded lettuce leaves, compared to the water treatment alone. Chlorine treatment is used in fresh-cut industry for preventing surface-borne microbes from being captured by stomata on leafy greens. Chlorine treatment appeared to be effective in disinfecting U.S. No.1 quality lettuce leaves, leading to a 2.8 log CFU/g reduction of *E. coli* counts compared to water-treated U.S. No.1 quality lettuce leaves ($p < 0.0001$). Meanwhile aqueous chlorine was as ineffective as potable water in reducing microbial loads on outgraded lettuce leaves, bringing about only 0.2 log CFU/g reduction of inoculated *E. coli*, compared to the water only treatment, due to deep internalization though the stems on damaged surfaces. It has been shown that cells can penetrate cut lettuce tissue to an average depth of 74 μm at 4°C and remain unaffected by treatment with chlorine (FDA, 2011). Because the rifampicin-resistant *E. coli* were inoculated on soil for carrots, the organic matters in soil limited the ability for chlorine to destroy microbes, and resulted in only a 0.4 log CFU/g *E. coli*

reduction on outgraded carrots, compared to the water only treatment.

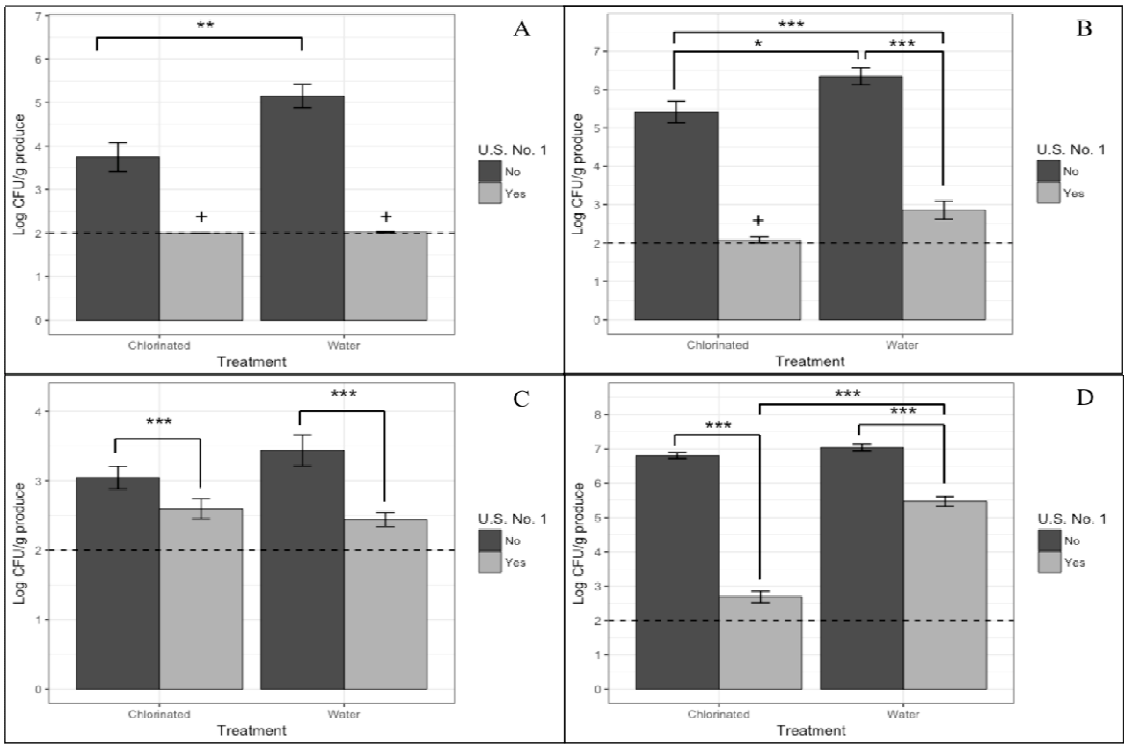

Fig. 1.2 Comparison between *Escherichia coli* counts on various produce with two different treatments. (A) Apple, (B) Tomato, (C) Carrot, (D) Lettuce. Symbols: Columns with a cross (+) were under limit of detection, so the corresponding category was not included in quantitative analysis; •p<0.1; *p < 0.05; **p < 0.01; ***p < 0.001.

1.4.3 Generic *E. coli* count disparities by grade

Produce was graded as "U.S. No.1", "Injury", "Damage" and "Serious Damage" according to the USDA grading standards. For each treatment, 25 U.S. No.1 and 25 outgraded (Injury, Damage, Serious damage) produce items were selected, but the numbers of each grade were not controlled. The log reduction of retained *E. coli* counts on each secondary quality grade, compared to counts on U.S. No.1 grade produce, are shown in Fig. 1.3. Water only and chlorine treatment left similar amount of generic *E. coli* counts reduction on apples graded as "Injury". Meanwhile inoculated *E. coli* counts reduction retained on damaged (p =0.03479) or seriously damaged (p =0.01199) apples were distinctly reduced by chlorine treatment compared to water only treatment (Fig. 1.3A). Injured and damaged tomatoes both received

about 1 log CFU/g reduction of *E. coli* from either water only or chlorine treatment; however, the *E. coli* reductions due to chlorine treatment was not significant on tomatoes of any grades, compared to water only treatment (Fig. 1.3B). This is because 80% outgraded tomatoes among all three grades carried open wounds, such as cuticle cracking. Chlorine treatment was as ineffective as potable water when disinfecting produce with open wounds, despite of the severity of wounds. Chlorine treatment gave a significant 1 log CFU/g difference on *E. coli* reduction between chlorine-treated and water-treated seriously damaged carrots ($p = 0.09308$), however, the efficacy of sanitizing was not significant on injured or damaged carrots (Fig. 1.3C). The inoculated *E. coli* counts reduction retained on lettuce leaves was significantly different between water only and chlorine treatments on outgraded lettuce leaves of all grades ($p < 0.001$). As mentioned previously, chlorine treatment significantly reduced the *E. coli* counts on U.S. No.1 quality lettuce leaves but not on outgraded lettuce leaves, and thus chlorine-treated U.S. No.1 grade lettuce leaves generally had a more distinct log reduction from outgraded lettuce leaves than did those following water only treatment (Fig. 1.3D). Despite the differences due to disinfection treatment, it is notable that inoculated *E. coli* counts retained on outgraded produce had no clear correlation with the suggested grades from the USDA Market Inspection Instructions.

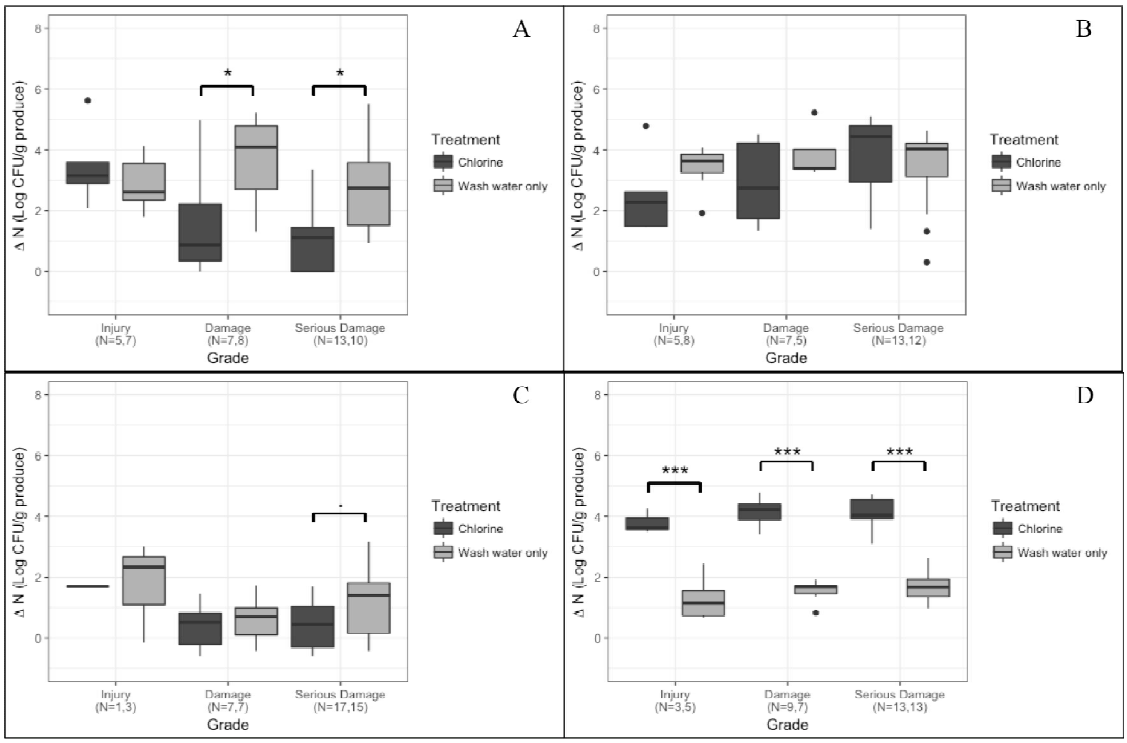

Fig. 1.3 Comparison between *Escherichia coli* count reductions following disinfection on various produce by grade. (A) Apple, (B) Tomato, (C) Carrot, (D) Lettuce. N indicates sample size, (N= number of sanitizer-treated samples, number of water-treated samples). •p<0.1; *p < 0.05; **p < 0.01; ***p < 0.001.

1.4.4 Generic *E. coli* counts disparities by type of deviation

Outgraded carrots and lettuce leaves were characterized by only one to two types of defects each, shape in the case of carrots and pink rib and physical damage in the case of lettuce. In contrast, the tomatoes and apples utilized in this study were subject to five to six different types of defects, which could be broadly categorized as either physiological defects or physical damage. Physical damage included any wound to the integrity of the surface of the fruit, as with bruises or broken cuticles, and cuts. Physiological defects included any wound remaining intact surface on produce, as with misshapes, rough patches or discoloration on cuticles. Because the outgraded produce was randomly selected, the numbers of each type of defect were not evenly distributed. The data showed that outgraded produce with physical damage was significantly more susceptible to bacterial retention following disinfection treatment than produce culled due to physiological defects ($p < 0.001$) (Fig. 1.4). Apples with

physical damage retained significantly more (2.6 log CFU/g) inoculated *E. coli* than did those with physiological defects ($p = 0.0031$) under chlorine treatment. No significant difference was observed between *E. coli* counts on water-treated apples between the two types of defect, but 0.8 log CFU/g more inoculated *E. coli* retention was observed on apples with physical damage. In addition, chlorine treatment significantly decreased *E. coli* counts by 1.6 log CFU/g on apples with physiological defects ($p = 0.0037$) compared to water only treatment. Meanwhile no significant reduction was obtained from chlorine treatment on outgraded apples with physical damage ($p = 0.764$) compared to water treatment. Tomatoes with physical damage had a significant 1.3 log CFU/g increase of retained *E. coli* counts compared to tomatoes with physiological defects following chlorine treatment ($p = 0.0301$). Similarly, an average of 0.6 log CFU/g more inoculated *E. coli* was retained on tomatoes with physical damage than those with physiological defects under water treatment, even though the difference was not statistically significant. The increased retention of pathogens following disinfection treatment suggested that produce with physical damage and physiological defects might actually represent different risk levels.

The assumption mentioned above holds true in the consideration of specific defects on outgraded produce. *E. coli* counts on apples with russeting were significantly ($p = 0.0037$) reduced by chlorine treatment due to the superficial defect characteristic (Table A1.1) No significant reduction was observed for any other type of defects, and meanwhile apples with open lesions barely had any reduction in *E. coli* at all (Fig. A1.1). In Fig. A1.2, it showed that retention of the *E. coli* counts significantly ($p = 0.0387$) increased on outgraded tomatoes with a growth crack defect following chlorine treatment compared to water only treatment. Deep cracks/holes extending through the tomato wall from the growth crack decreased the efficacy of sanitizers further. In addition, growth cracks appeared to trap the highest *E. coli* counts following both water only and chlorine treatment. It has been reported that growth cracks provided a better protective environment for bacteria against treatment with water or aqueous chlorine (Wei et al., 1995). Injuries, such as bruises, did not retain as many pathogens as the other defects; however, such defect might induce secondary

infections that flavor growth of pathogens (Wells & Butterfield, 1997).

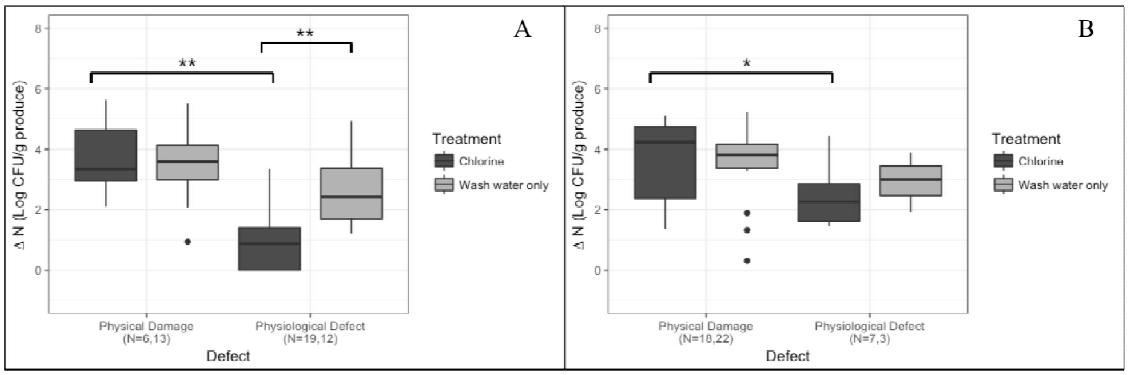

Fig. 1.4 Comparison between *Escherichia coli* count reductions following disinfection by type of defect. (A) Apple, (B) Tomato. N indicates sample size, (N= number of sanitizer-treated samples, number of water-treated samples). •p<0.1; *p < 0.05; **p < 0.01; ***p < 0.001.

1.5 Discussion

The impact of using secondary quality or outgraded produce on the retention of surface-inoculated *E. coli* following disinfection treatment was evaluated on four model fresh produce systems (apple, tomato, carrot, lettuce). Generally, outgraded produce of all four kinds retained significantly more inoculated *E. coli* counts following both water only and chlorine treatments. With a growing interest in directing secondary quality produce to the fresh market, outgraded produce could potentially increase the food safety risks upon associated with fresh produce consumption. Application of chlorine treatment was not sufficient to eliminate the additional pathogen retention on secondary quality or outgraded produce. The increased retention of pathogens following disinfection treatments should be considered in risk assessments on the introduction of secondary quality produce into the fresh market.

Outgraded produce is often characterized by structural and surfaces deviations (cracks, crevices, hydrophobic tendency, texture). This not only creates niches, which trap contaminants, but may also decrease the efficacy of sanitizers weakened by organic matter from damaged fruit/vegetable tissue (Felkey et al., 2006). Previous studies have shown that different types of sanitizers were variably affected by produce surface harborage sites (Yuk et al., 2005; Alvarado-Casillas et al., 2007). Intact plant

cell walls provide an effective barrier against the internalization of microbes (Zhang & Zhou, 2010). Pathogens can more readily penetrate through fresh wounds or decayed areas in produce, and sanitizers have limited ability to destroy pathogens once they are internalized (Fatemi & Knabel, 2006; Fatemi et al., 2006). In the study, a chlorine dip treatment significantly reduced surface-inoculated *E. coli* counts on outgraded apples and tomatoes, whereas the treatment had little effect on pathogen inactivation on outgraded lettuce leaves due to deep internalization through the stems on damaged surfaces. Fresh leafy vegetables with punctures, cuts or decayed areas retain high microbial loads following decontamination. Moreover, once bacteria penetrate the interior of a fruit or vegetable, they increasingly withstand diverse decontamination treatments, such as surface pasteurization with hot water or steam for blanched, frozen produce (Sapers, 2003). Simply increasing the concentration or exposure time for sanitizer treatments to compensate for interference from organic matter has its drawbacks as it may cause unacceptable sensory changes on the produce without significantly increasing lethality (Snyder et al., 2016). Therefore, the practice of culling defective leaves from the packing or processing line could help minimize food safety risks.

Currently, fresh produce is culled for quality defects; however, some quality defects may also be associated with food safety hazards. Determination of produce marketability relies on the suggested grades from the USDA Market Inspection Instructions or market requirements. *E. coli* counts retained on outgraded produce in this study had no clear correlation with the suggested grades for a given defect. The *E. coli* levels on outgraded tomatoes and lettuce leaves suggested that a chlorine dip treatment was as ineffective as potable water when disinfection produce with open wounds, regardless of the severity of damage. In order to maximize food resources, a scheme for fresh market acceptance should encompass the relative food safety risk for a given defect.

The minimum quality standards set by the federal marketing orders do not distinguish culled produce with intact surfaces from those lacking surface integrity. Culls due to misshaped or malformed produce, wrong sizes (too large, too small) or

superficial color defects are often discarded or left in the field among those with open lesions or bruises. The data presented here suggests that these two deviations, physiological defect and physical damage, do not represent equivalent food safety risks as evaluated by retention of *E. coli.* In this study, apples and tomatoes were diagnosed with five to six different types of defects. These pathological issues were further categorized into two broad categories - physiological defect (intact surface) or physical damage (lacking surface integrity). The increase load of retained *E. coli* on outgraded apples and tomatoes with physical damage agreed with previous studies showing that chlorine treatments were less effective in reducing pathogen levels on produce flesh than those with intact surfaces (e.g. Zhuang et al., 1995; Han et al., 2001). However, in this study, that comparison extended to outgraded produce with other defects besides physical damage. The levels of *E. coli* varied by type of deviation, and showed that outgraded produce with physical damage is significantly more susceptible to bacterial retention following disinfection than produce culled due to physiological defect. This suggests that culled produce with physical damage and physiological defects might actually represent different risk levels and should be evaluated differently for introduction into the fresh market. This discrepancy is likely due to the presence of organic matter from exposed produce flesh, which decreases the efficacy of antimicrobials.

Additionally, this study only focused on pathogen reduction through the disinfection step, but pathogen contamination levels might be further exacerbated through growth of pathogens on physically damaged sites during cold storage. Besides decreasing efficacy from application of antimicrobials, the exposure of plant nutrients on the physically damaged sites may facilitate growth of pathogens throughout shelf life. Previous studies have shown that apples with cuts or wounds that are contaminated before refrigeration support prolonged survival of pathogens, and postharvest decontamination process, such as chlorine treatment, do not fully prevent from bacterial regrowth due to inaccessible protected sites for pathogens (Han et al., 2002; Wei et al., 1995). These results suggest that using outgraded produce with physical damage could potentially introduce higher food safety risks associated with

fresh produce consumption than did those with physiological defects. Therefore, directing physically damaged produce away from the fresh market is a risk management strategy against contaminated product reaching the consumer. This compromise, where physiological defects and physical damage are treated as distinct, may be a worthwhile tradeoff in addressing food waste.

In conclusion, these findings show that outgraded produce retains more generic *E. coli* than U.S. No.1 grade produce after contamination, and that treatment with aqueous chlorine (150 ppm) was not sufficient overcoming this increased retention. Disinfecting produce with food grade sanitizers mitigates the food safety risks on consuming outgraded produce by limiting cross-contamination and providing a modest (1-2 log reduction) in pathogens (Sapers, 2001; Fatemi & Knabel, 2006). However, the type and degree of damage impacts the efficacy of sanitizers. Outgraded produce with physiological defects poses less risk than does that with physical damage. Pathogen proliferation on damaged produce surfaces likely increases the food safety risks associated with produced culled due to physical damage. Therefore, as industry considers how to minimize its food waste problem, preferentially directing physically damaged produce away from the fresh market will help to minimize risk while utilization of produce with physiological defects may contribute to food waste reductions.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

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APPENDIX A

Table A1.1: Produce grading rubric based on the USDA Market Inspection Instructions (USDA, 2004 & USDA, 2005). Grading scheme was used in assigning categorical quality scores to fresh produce.

	Apple	Tomato	Carrot	Lettuce
U.S. No. 1	"U.S. No. 1" consists of apples which are mature but not overripe, clean, fairly well formed, and free from decay, internal browning, internal breakdown, soft scald, freezing injury, and **broken skins**. The apples are also free from damage caused by **bruises**, **brown surface discoloration**, sunburn or sprayburn, limb rubs, hail, drought spots, scars, stem or calyx cracks, disease, **insects**, bitter pit, Jonathan spot, or damage by other means.	"U.S. No.1" consists of tomatoes which are mature but not overripe or soft, clean, **fairly well formed**; which are free from decay, sunscald, and freezing injury, and free from damage caused by **bruises**, **cuts**, shriveling, puffiness, **catfaces**, growth **cracks**, **scars**, disease, **insects**, or other means	"U.S. No. 1" consists of carrots which are well trimmed, firm, fairly clean, fairly well colored, **fairly smooth**, **fairly well formed**; which are free from soft rot, and free from damage caused by freezing, growth cracks, sunburn, pithiness, woodiness, internal discoloration, oil spray, dry rot, other disease, insects or other means.	"U.S. No. 1" consists of heads of lettuce which are fresh, green, not soft, not burst, **free from decay**, doubles, fairly well trimmed, and not damaged by any other cause.
Injury	• **Brown surface discoloration (scald)** > ¼ inch in diameter. • **Russeting** smooth net-like >10% surface area • **Bruises** >1/8 inch in depth or 5/8 inch in diameter. • **Broken skins and cuts**: any healed skin break. • Any **healed sting** total area > 1/8 inch in diameter. • Apple is **not seriously deformed.**	• Healed **cut** > ½ inch. • **Catface scars** aggregating > a circle ½ inch in diameter. • Not well healed **cracks** > 1/8 inch in depth or > ½ inch in length. • **Insect stings** aggregate more than 3/8 inch in diameter. • Aggregated **zipper** length exceeds the length from the outer edge of the stem scar to the blossom end of the tomato. • Tomato is **not fairly well formed**. • Tomato of which more than 3/8-inch aggregated area is affected by **bruising**.	• Carrot that is **not forked, or misshapen** to the extent that its appearance is materially affected.	• **Physical damage or pink rib** occurs on any leaf.

Damage	• **Brown surface discoloration (scald)** >1/2 inch in diameter. • **Russeting** smooth net-like >15% surface area; rough >1/4 inch in diameter • **Bruises** >3/16 inch in depth or 7/8 inch in diameter. • **Broken skins and cuts**: healed skin >1/4 inch in diameter or 1/8 inch in depth. • Any **healed sting** total area > 3/16 inch in diameter. • Apple is **slightly deformed.**	• Healed **cut** > ½ inch. • **Catface scars** aggregating > a circle 3/4 inch in diameter. • Not well healed **cracks** > 1/8 inch in depth or > 3/4 inch in length. • **Insect stings** aggregate more than 5/8 inch in diameter. • Aggregated **zipper** length exceeds more than 2 times the length from the outer edge of the stem scar to the blossom end of the tomato. • Tomato is **not reasonable well formed**. • Tomato of which more than 5/8-inch aggregated area is affected by **bruising**. •	• Carrot that is **forked, or twisted, curved or, otherwise ill-formed** to the extent that the appearance is materially affected.	• **Physical damage** and **areas of deep pink color** more than 2-inches in length.
Serious damage	• **Brown surface discoloration (scald)** > ¾ inch in diameter. • **Russeting** smooth solid >50% surface area • **Bruises** >3/8 inch in depth or 1-1/8 inch in diameter. • **Broken skins and cuts**: healed skin >1/2 inch in diameter & any unhealed skin. • Any **healed sting** total area > 1/4 inch in diameter. • Any **immature or overripe** fruit. • Apple is **seriously deformed.**	• Fresh **cut** or healed and extending through the tomato wall. • **Catface scars** aggregating > a circle 1 inch in diameter. • Not well healed **cracks** > 1/4 inch in depth or > 1 inch in length. • **Insect stings** aggregate more than 1 inch in diameter. • Any **insect** in the fruit, or **feeding injury** which extends through the wall or into the interior of the tomato. • **Zipper** holes or channels occur along the scar to a greater extent	• Carrot that is **misshapen** to the extent that the appearance is seriously affected.	• **Physical damage** or **areas of deep pink color** seriously detracts from the appearance of the edible quality.

		than allowed for other scars and or catfaces. • Tomato that is **badly misshapen**. • Tomato of which more than 3/4-inch aggregated area is very seriously affected by **bruising**, and/or that are cracked or split open.		
Physiological defects	• **Brown surface discoloration (scald)** • **Russeting** • Apple is **not fairly well formed.**	• **Zipper** • Tomato is **not fairly well formed.**	• Carrot is **not fairly smooth, not fairly well formed.**	• None
Physical damage	• Any **healed sting** • **Bruises** • Any apple with **broken skins and cuts**	• **Insect stings** • **Bruises** • **Catface scars** • **Growth cracks** • **Healed/unhealed cut**	• None	• **Physical damage or areas of deep pink color**

Table A1.2: Summary of bacterial counts under/above the limit of detection (LOD)

		Water Treatment	Chlorination Treatment
Apple	U.S. No. 1	All under LOD except one	All under LOD
	Outgraded	All above LOD	Mixed
Tomato	U.S. No. 1	Mixed	All under LOD except one
	Outgraded	All above LOD	All above LOD
Carrot	U.S. No. 1	Mixed	Mixed
	Outgraded	Mixed	Mixed
Lettuce	U.S. No. 1	All above LOD	Mixed
	Outgraded	All above LOD	All above LOD

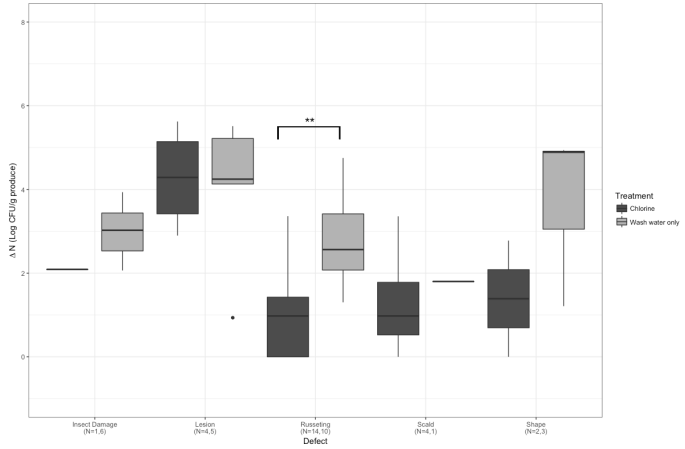

Fig. A1.1 Comparison between *E. coli* count reductions following disinfection on apples by specific defect. N indicates sample size, (N= number of sanitizer-treated samples, number of water-treated samples). •p<0.1; *p < 0.05; **p < 0.01; ***p < 0.001.

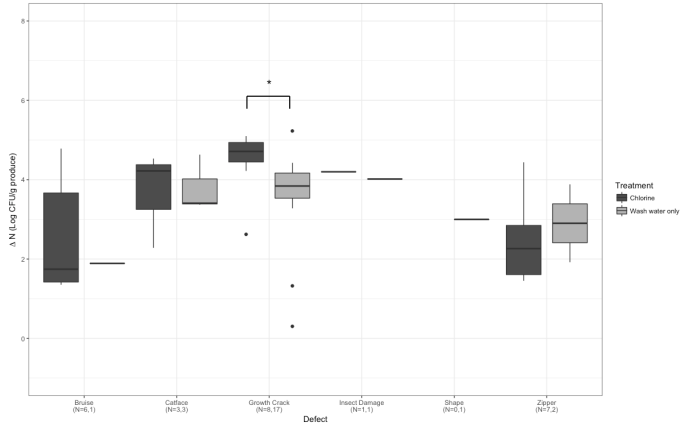

Fig. A1.2 Comparison between *E. coli* count reductions following disinfection on tomatoes by specific defect. N indicates sample size, (N= number of sanitizer-treated samples, number of water-treated samples). •p<0.1; *p < 0.05; **p < 0.01; ***p < 0.001.

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CHAPTER 2
COMBINED EFFECT OF TEMPERATURE ABUSE, SURFACE INTEGRITY, AND LENGTH OF SHELF LIFE ON THE GROWTH OF *LISTERIA MONOCYTOGENES* AND SPOILAGE MICROBIOTA FOR REFRIGERATED READY-TO-EAT PRODUCTS

2.1 Abstract

The inconsistency of date labels is considered to be one of the causes of food waste. The public does not always understand the myriad of date labels including "Used-by", "Sell-by" or "Best-before", so are unsure when food is safe to consume or should be discarded. Industries are trying to standardize date labeling regulations, but in the absence of standard labeling, this confusion leads to increased food waste. The purpose of this study is to evaluate the interactions of quality attributes and *Listeria monocytogenes* growth on six model ready-to-eat (RTE) product systems (tomatoes, apples, fresh-cut cantaloupe slices, fresh-cut lettuce leaves, baby spinach leaves, commercially processed turkey slices) under different refrigeration temperatures. The growth of both inoculated *Listeria monocytogenes* and spoilage microbiota was monitored on select RTE products throughout the shelf life of the different products. Generally, when any of the hurdles to bacterial growth are disrupted, *L. monocytogenes* growth becomes problematic before the quality of food deteriorates under both strict and abuse refrigeration temperatures. Whereas rapid growth of spoilage microbiota limits the shelf life of outgraded produce with physical damage, and thus makes the survival and growth of retained pathogens, during post-harvest storage, less relevant to food safety. Therefore, select U.S. No. 1 grade fruit (tomatoes, apples), along with the culls, pose less food safety risks from *L. monocytogenes* growth under refrigerated storage compared to the fresh-cut products.

2.2 Introduction

According to the Food and Agriculture Organization of the United Nations (2011) on global food losses and food waste, more than 40% of food waste occurs at

the end of the food chain (i.e., retail and final consumption) in developed countries. One cause of food waste is the confusion and misinterpretation of date labels amongst consumers. Date labels, such as "Used-by", "Sell-by" or "Best-before", are largely unregulated and not always indicative of food safety risks. Approximately 25% of household food and beverages are discarded because they were past expiration dates, the production code was misinterpreted, or they were otherwise deemed too old (NRDC, 2012). Inconsistent usage of date labels promotes uncertainty and food waste.

Currently, date labeling is not required by federal regulations in the United States, except for infant formula, which is due largely to nutrient loss, as opposed to microbial pathogen growth (Newsome et al., 2014). Both the U.S. Food and Drug Administration (FDA) and U.S. Department of Agriculture (USDA) have date labeling policies, causing inconsistent regulations for manufacturers and misleading information for consumers. The U.S. FDA does not require food companies to place any date labels on food products. Meanwhile, the USDA claims that a "Best if Used by" date is best understood as a quality date (USDA-FSIS, 2016). However, the USDA updated food product dating guidance does not make suggestions toward a standardized approach for companies to establish said date. On one hand, some advocacy groups want to eliminate date label regulations all together but until that happens they are encouraging the public to use food that has passed the date label to reduce waste, which might be acceptable on condition that the food safety is not compromised. On the other hand, industries are trying to standardize date labeling regulations to achieve the balance between food safety and waste reduction. (NRDC, 2013; ReFED, 2016)

It is generally agreed that date labels most often indicates whether the food is no longer at peak quality. However, the "Use-By" labels for refrigerated ready-to-eat (RTE) foods are additionally associated with food safety cut-offs, based on the possible survival and growth of psychrotolerant bacteria at refrigeration temperatures. This is a significant concern since some date labels are just for quality and some are for safety but this is currently not clearly conveyed to consumers. Psychrotolerant bacteria, such as pathogenic microorganisms *Clostridium botulinum* type E, *Listeria*

monocytogenes, *Yersinia entercolitica*, enterotoxigenic *Escherichia coli* and *Aeromonas hydrophila* grow at or below 6°C (Robertson, 2005). Should the psychrotolerant bacteria grow on refrigerated RTE food at refrigeration temperatures, higher levels may be ingested by consumers since there is no kill step prior to consumption. FAO/WHO (2004) estimated the lethal dose of *L. monocytogenes* to be 1.9×10^6 CFU for human listeriosis. For pathogens with high infectious dose, growth during refrigerated storage can increase the risk of foodborne illness.

Amongst the psychrotolerant pathogens, *L. monocytogenes* appears to be one of the leading causes of hospitalizations and deaths due to foodborne illnesses. The Center for Disease Control (CDC) estimated that 1,600 people get listeriosis each year, and approximately 260 die in the United States. Listeriosis outbreaks were primarily linked to the consumption of deli meats and hot dogs in the 1990s, and today, are often associated with dairy products and fresh produce (CDC, 2017). In a recent multistate outbreak involving contaminated bagged cut salads, 1 death and 19 hospitalizations resulted (CDC, 2016). In a Canadian outbreak in 2008, deli meat contaminated from slicing machines caused 57 cases of serious illnesses from listeriosis and 23 deaths (Currie et al., 2015). *L. monocytogenes* is commonly recognized as a high-risk organism for people with weakened immune systems, and thus the FDA has zero tolerance on the presence of *L. monocytogenes* in food products.

Temperature abuse during cold storage has been associated with increased *L. monocytogenes* growth. It has been reported by Jol et al. (2005) that 20% of the domestic refrigerators are found to operate at higher than 10°C. If consumers are instructed to ignore, or industry decides to eliminate date labels from these types of products, the high levels of *L. monocytogenes* resulting from long-term storage may pose an increased food safety risk. Studies have shown that *L. monocytogenes* survive and remain viable under strict refrigeration, and grow at elevated temperatures on RTE products, so in addition to understanding the labels, consumers should be made aware of storage temperatures required to maintain safety (Steinbrugge et al., 1988; Beuchat and Brackett, 1990a; Vandamm et al., 2013).

Moreover, fruit and vegetable with cut wounds may provide *L. monocytogenes* a more favorable growth environment due to the disruption of the intact skin. Previous studies have identified that the growth of *L. monocytogenes* is accelerated on damaged surfaces on fresh produce (Table. A2.1). Subsequently damaged produce of secondary quality may preferentially harbor microbial pathogens, and support their proliferation in the case of open lesions under abuse refrigeration temperature. The food safety problem caused by a long shelf life for RTE foods may be exacerbated by the additional use of culls to reduce food waste. Additional barriers, such as modified atmosphere packaging (MAP), are usually applied to processed foods as a means to control microbial growth. The behavior of *L. monocytogenes* under modified temperatures at strict and abuse refrigeration temperatures is well studied for meat, poultry, seafood or for whole vegetables, while less is known about the combined preservative effect of storage temperature and packaging atmosphere on fresh-cut fruits and vegetables and the interface between safety and quality (Table. A2.1). Quality management programs, such as strict temperature control and modified atmosphere packaging, keep products at peak quality for a longer time. However, these strategies may not as readily control the growth of psychrotolerant and facultative anaerobic *L. monocytogenes*, and thus creating a favorable environment for elevated growth during long storage periods. Consumers are instructed to use signs of spoilage as fail-safe indicators in terms of shelf-life limitation for *L. monocytogenes* growth. Therefore, increased preservation on food quality that does not impact *L. monocytogenes* may lead to more serious food safety problems.

The competing interests among quality, safety, and food waste not only intersect, but have unintended consequences in the marketplace if any one them is neglected. In order to standardize date labeling regulations and reduce food waste, risk factor identification and proper risk management strategies must be implemented to ensure food safety. It is also critical that this information be shared with consumers so they understand the labeling. This study was conducted to study the interactions of quality attributes and *Listeria monocytogenes* growth on six model RTE product systems stored at different refrigeration temperatures as a foundation for a safety-

based shelf life date label.

2.3 Materials & methods

2.3.1 Bacterial strains & inocula preparation

A three-strain cocktail of *L. monocytogenes* was used to inoculate RTE products. The cocktail for fresh fruits and vegetables contained SL R9-0506 1/2a [II], SL R9-5411 1/2b [I] and FSL R9-5506 4b [I]. These strains were originally isolated from the 2011 cantaloupe outbreak (CDC, 2012), 2015 caramel apple outbreak (CDC, 2015) and 2016 packaged salad mix outbreak (CDC, 2016), respectively. The cocktail for turkey slices contained Scott A (FSL J1-225), H7858 (F6-366) and J0161 (FSL R2 499). These strains were originally isolated from pasteurized milk, RTE meat and delicatessen sliced turkey outbreaks (Roberts, 2009; Nelson et al., 2004). Bacterial cultures were stored at -80°C in Tryptic Soy Broth (TSB) (Becton, Dickinson and Co., Sparks, MD) containing 25%(vol/vol) glycerol. To prepare the inocula, the three *L. monocytogenes* strains were grown separately in 5 mL TSB and incubated at 30°C for 18 h on a rotary shaker (200 RPM). After incubation, *L. monocytogenes* cells were harvested by centrifugation (13,000 RPM, 10 minutes), washed twice and re-suspended in phosphate-buffered saline (PBS, pH 7.0, Fisher Chemical, Inc., Fair Lawn, NJ). The three bacterial suspensions were combined at equal volumes and diluted with PBS by 4-fold dilution. The final concentration of the inocula was determined by plating on *Listeria monocytogenes* Chromogenic Plating Medium plates (LMPM) (Becton, Dickinson and Co., Sparks, MD) to be 5.23±0.16 log CFU/ml.

2.3.2 Sample preparation

Six commodities, cantaloupe (Infinite Gold, purchased from a commercial retailer), tree-picked apples (Red Delicious, Cornell University, Ithaca, NY), ripe-picked tomatoes (BHN 589, Cornell University, Geneva, NY), baby spinach leaves (Red Cardinal, purchased from a commercial retailer), lettuce leaves (Romaine, purchased from a commercial retailer) and vacuum-packed sliced turkey meat (oven-baked turkey breast, purchased from a commercial retailer) were selected to represent

a wide variety of common RTE products on the market. Ripe-picked tomato samples included U.S. No.1 quality and culled tomatoes. Culled tomatoes were separated into two categories based on surface integrity. Culled tomato samples with intact surfaces included defects like zippers, and non-standard size and shapes. Culled tomato samples with open lesions included defects, such as growth cracks and cuticle cracking. All the selected produce items were held at 4°C for up to 48 h until use.

Cantaloupe and Red Delicious apples were cut into equally sized pieces of about 300 mm^2 with 4-mm thickness using sterile knives. Lettuce leaves were cut into equally sized squares about 300 mm^2 with sterile knives. Red Delicious apple slices were dipped in anti-browning solution with 1% citric acid and 0.5% ascorbic acid (Sigma Chemical Co., St. Louis, MO) to simulate industrial practices. The anti-browning solution (pH 5.37) appears to be less acidic than Red Delicious apple juice (pH 4.4).

The initial counts of *L. monocytogenes* and spoilage microbiota were evaluated in triplicate 2 hours after inoculation. Controlled samples without inoculation were free of background *L. monocytogenes* contamination.

2.3.3 Inoculation

Ten grams of either fresh-cut cantaloupe slices, Red Delicious apple slices, lettuce leaves, baby spinach leaves and turkey slices were weighed and spot-inoculated with 10 × 10-uL aliquots of the *Listeria monocytogenes* cocktail inocula. Intact surfaces of tree-picked apples and U.S. No.1 quality tomatoes were spot-inoculated with 10 ×10-uL aliquots of the *Listeria monocytogenes* cocktail inocula. A 2-inch×2-inch square was marked with permanent markers for later identification of all inoculation sites. Culled tomatoes were inoculated on the sights of damage or defect. A mixed inoculation sites of intact surfaces and cut wounds on fresh-cut lettuce leaves under atmospheric storage and the inocula were allowed to air dry for 2 h, at ambient temperature, prior to storage.

2.3.4 Packaging and storage of samples

Inoculated fresh-cut cantaloupe slices, Red Delicious apple slices, lettuce leaves, baby spinach leaves and turkey slices were packaged in polypropylene films (Printpack Inc., Alanta, GA) to simulate commercial packaging conditions. Fresh-cut cantaloupe slices, Red Delicious apple slices and baby spinach leaves were packaged in polypropylene monoweb with perforations having an oxygen transmission rate (OTR) of 15000+ $mL/m^2/day/atm$ to accommodate high respiration rates and low tolerance of reduced oxygen. Fresh-cut lettuce leaves were packaged in polypropylene adhesive laminated to polyethylene without perforations having an OTR of 1395 $mL/m^2/day/atm$. Romaine lettuce bags are flushed with nitrogen at packing for 20 seconds and reached an initial level of 6.85±0.38% oxygen, in order to simulate commercially MAP condition. Theoretically, the respiration of the product continues to reduce the modified atmosphere further until it is essentially 0% O_2 and 6-10% CO_2. A gas analyzer (Mocon Inc., Minneapolis, MN) was used to monitor the gas composition changes in Romaine lettuce packages. Turkey slices were packaged in high barrier films with an OTR of 0.5 $mL/m^2/day/atm$. BD GasPak™ EZ Anaerobic system (Becton, Dickinson and Co., Sparks, MD) was used to simulate anaerobic MAP packaging conditions of deli meat.

Samples were stored in refrigerators at 4±1°C and 8±2°C. The 8°C temperature was chosen to simulate temperature abuse in distribution and household refrigeration. The length of storage was determined based on the common shelf life of each product. Fresh-cut cantaloupe slices were stored for five days and evaluations were made in triplicate every 24 hours. Apple slices were stored for 12 days and sampled in triplicate every 48 hours. Tree-picked apples, tomatoes, packaged fresh-cut Romaine lettuce leaves, and packaged baby spinach leaves were stored for 27 days and sampled in triplicate twice per week. Packaged turkey slices were stored for 70 days and sampled in triplicate once per month.

2.3.5 Recovery and enumeration of bacteria

The inoculated sections of whole fruits were excised with sterile knives and

transferred directly into sterile stomacher bags (Whirl-Pak, Nasco, Jackson, WI). Ten grams of packaged products were transferred directly into stomacher bags. Samples were 10-fold diluted with PBS based on weight and homogenized in a stomacher (STOMACHER® 400 CIRCULATOR) for two mins at 230 RPM. Filtered homogenates were diluted and spiral-plated onto LMPM plates using an Autoplate 4000 (Spiral Biotech, Inc., Norwood, MA) for *L. monocytogenes* detection. Spoilage microbiota were detected by spiral-plating diluted homogenates onto Potato Dextrose Agar plates (PDA) (Becton, Dickinson and Co., Sparks, MD) for fruit samples, on Crystal Violet Tetrazolium Agar plates (CVTA) (Becton, Dickinson and Co., Sparks, MD) for leafy green samples, and de Man, Rogosa and Sharpe Agar plates (MRS) (Becton, Dickinson and Co., Sparks, MD) for meat samples. LMPM agar plates were incubated at 35°C for 48 hours. CVTA plates were incubated at 21°C for 48 hours. PDA plates were incubated at 25°C for 48 hours. MRS plates were incubated at 30°C for 48 hours. Colonies were enumerated with the Colony Counter (Spiral Biotech, Inc., Norwood, MA) after incubation. The 1-log growth mark was established as a critical limit where *L. monocytogenes* growth became problematic for holding cold food without temperature control from a safety perspective (USPHS, 2013). The growth rates of *L. monocytogenes* and spoilage microbiota were summarized in Table 2.1 based on said critical limits. The evaluation of quality was mainly based on smell, appearance, and texture, and carried out by one researcher. The deviations included surface discoloration, fungal growth, off-odor and sliminess. To account for the limited number of panelists in this experiment, the counts of spoilage microbiota, as referred to in previous literature, were utilized when assessing the sensory attributes. Whether the detectable signs of spoilage appeared on select foods can also be found in Table 2.1.

2.3.6 Statistical analysis

All statistical analyses were performed in R (version 3.3.1, R studio, Boston, MA). A two-way ANOVA test was performed to evaluate statistically significant differences between the average log growths of *L. monocytogenes* under 4°C and 10°C

on fresh-cut cantaloupe, fresh-cut apple slices and baby spinach leaves. Separate models were used for each food type. Pairwise comparison between *L. monocytogenes* counts under two temperatures based on sampling days were evaluated using least square means (lsmeans) package (Lenth, 2016). A three-way ANOVA test was performed to evaluate statistically significant differences among the average log growths of *L. monocytogenes* at 4°C and 10°C in two different packaging conditions (atmospheric & modified atmosphere storage) on commercially processed turkey slices and fresh-cut lettuce. Separate models were used for each food type. A three-way ANOVA test was also performed to evaluate statistically significant differences among the average log growths of *L. monocytogenes* at 4°C and 10°C with different produce surface integrities on tree-picked apples and ripe-picked tomatoes. Separate models were used for each food type. Pairwise comparison of interactions between temperatures and storage conditions/surface integrities based on sampling days were evaluated using least square means.

2.4 Results

2.4.1 *L. monocytogenes* and spoilage microbiota growth on various fresh-cut produce

Fresh-cut cantaloupe has a shelf life of up to three days and is usually sold in plastic clamshell containers. Even though Gorny (1997) recommended modified atmosphere for fresh-cut cantaloupe cubes, MAP is not commonly practiced at retail stores with regards to fresh-cut cantaloupe packaging. In this study, fresh-cut cantaloupe samples were stored at 4°C and 10°C with atmospheric storage for five days. Fresh-cut cantaloupe slices had a 0.90 log CFU/g increase of *L. monocytogenes* counts after two days at 4°C, while it only took one day for *L. monocytogenes* to reach a 1.05 log CFU/g increase when stored at 10°C (Fig. 2.1). Fresh-cut cantaloupe stored at 4°C had a 1.19 log CFU/g increase of yeast counts after two days of storage. Meanwhile cantaloupe samples stored at 10°C had a 2.49 log CFU/g increase of yeast counts after two days, leading to a total yeast counts of 5.9 log CFU/g (Fig. 2.1). Jacxsens et al. (1999) reported that consumers could usually detect sensory deterioration of minimally processed fruits and vegetables when the count of yeast and

molds reaches levels above 5 log CFU/g. Yeast growth in this study reached the sensory threshold determined by Jacxsesns et al. (1999) within five days on fresh-cut cantaloupe stored under strict refrigeration, whereas it only took two days for inoculated *L. monocytogenes* to reach a 10-fold increase on fresh-cut cantaloupe under these same conditions. In addition, the results from the two-way ANOVA test showed that strict refrigeration temperature significantly inhibited the growth of *L. monocytogenes* after 24 hours of storage and remain significant throughout shelf-life (five days of storage) compared to abuse refrigeration temperature ($p < 0.0001$).

Fresh-cut apple has a shelf life of up to 10 days, and are usually packed in micro-perforated film with no gas flushing at packing due to its intolerance of low oxygen and higher respiration rate (Holcroft, 2017). Therefore, fresh-cut apple samples treated with anti-browning agents were stored at 4°C and 10°C in micro-perforated film with atmospheric storage for 12 days to simulate industry practices. A more than 10-fold (1.3 log CFU/g) increase of *L. monocytogenes* occurred on fresh-cut apple slices after six days of storage at 4°C (Fig. 2.5C). Meanwhile the samples stored at 10°C had a 2.84 log CFU/g increase of *L. monocytogenes* after four days. Yeast growth on fresh-cut apple slices increased by 4.55 log CFU/g after four days for samples under abuse refrigeration temperature and by 2.24 log CFU/g after six days for those under strict refrigeration (Fig. 2.5C). No sign of spoilage was observed from apple slices stored at 4°C, and meanwhile a 10-fold increase of *L. monocytogenes* was observed after six days. In addition, the log growths of *L. monocytogenes* on fresh-cut apple slices were significantly different between samples stored at 4°C and 10°C after 4-10 days ($p < 0.01$).

Bagged baby spinach has a shelf life of up to 16 days and is commercially packed in micro-perforated film or rigid clamshells with minor atmosphere modifications. In this study, store-bought baby spinach leaves were stored at 4°C and 10°C for 27 days in micro-perforated film with atmospheric storage. The log growth of *L. monocytogenes* increased by 1.43 log CFU/g after 20 days of storage at 4°C and 0.98 log CFU/g after two days of storage at 10°C (Fig. 2.2C). The log growth of Gram-negative bacteria on baby spinach leaves increased by 2.63 log CFU/g after 20

days of storage at 4°C and 1.57 log CFU/g after 2 days of storage at 10°C (Fig. 2.2C). Babic et al. (1996) reported that the texture of the fresh-cut spinach leaves decreased significantly between days two and five when stored at 10°C. This study showed that the entire bag of baby spinach leaves was subject to soft rot as dextran (slime) was produced by spoilage bacteria after 20 days of storage at 10°C. However, when stored at 4°C, the sensory attributes remained acceptable throughout the entire experiment (27 days) based on the quality evaluation of appearance and texture. The growth of *L. monocytogenes* was significantly higher on spinach leaves stored at 10°C than that at 4°C after 2-15 and 22 days of storage ($p < 0.05$).

A three-way ANOVA test was performed to evaluate statistically significant differences between average log increases *L. monocytogenes* at 4°C and 10°C on fresh-cut lettuce leaves with atmospheric storage and modified atmosphere (MA) storage. Bagged Romaine lettuce has a shelf life of up to 16 days and is usually packed in low-OTR films with passive modified atmosphere packaging method by nitrogen flushing to reduce oxygen level to 4-8%. In this study, fresh-cut lettuce leaves were stored under both atmospheric and modified atmosphere to simulate common industry and household practices. Lettuce leaves were stored at 4°C and 10°C for 27 days. Inoculated *L. monocytogenes* grew on fresh-cut lettuce leaves under MA storage increased by 1.14 log CFU/g after 20 days at 4°C and 0.98 log CFU/g after two days at 10°C (Fig. 2.2A). *L. monocytogenes* on atmospheric-stored lettuce leaves increased by 0.06-log CFU/g after 27 days at 4°C and by 0.98 log CFU/g after 6 days at 10°C (Fig. 2.2B). The growth of Gram-negative bacteria reached greater than 2.1 log CFU/g by the time the growth of *L. monocytogenes* increased by 10-fold or at the end of the experiment on lettuce leaves under all experimental conditions, except for the samples with MA storage at 10°C. The Gram-negative bacteria on lettuce leaves packaged under modified atmosphere and stored at 10°C increased by 0.92-log CFU/g after two days (Fig. 2.2A). The log growth of *L. monocytogenes* on atmospheric-stored fresh-cut lettuce leaves under abuse refrigeration temperature were significant higher than that under strict refrigeration temperature after 6-27 days of storage ($p < 0.05$). Similarly, the log growth of *L. monocytogenes* on MA-stored fresh-cut lettuce leaves under abuse

refrigeration temperature was significantly higher than that under strict refrigeration temperature after 18-22 days of storage ($p < 0.05$). Signs of spoilage on MA-stored samples appeared after 27 days, and atmospheric-stored apples appeared after 9 days of storage at 10°C. Whereas the log counts of gram-negative bacteria on samples stored at 4°C remained less than 10^7 CFU/g and showed no signs of spoilage throughout the entire experiment. Subsequently, the signs of spoilage appeared later than the time when *L. monocytogenes* reached a 10-fold increase under all experimental conditions on fresh-cut fruits and vegetables in this study.

2.4.2 *L. monocytogenes* and spoilage microbiota growth on commercially processed turkey slices

Deli meat, which is commonly stored in modified atmosphere packaging, was also tested as a model for evaluating the combined impact of storage temperature and storage conditions on *L. monocytogenes* and spoilage microbiota growth. A three-way ANOVA was performed to evaluate statistically significant differences between average log increases of *L. monocytogenes* at 4°C and 10°C on turkey slices with atmospheric and saturated CO_2 atmosphere. Pre-packaged deli meat made and packaged in a USDA-inspected processing plant has a shelf life of up to 70 days and is usually packed in a modified atmosphere packaging (30% CO_2 and 70% N_2) with high-barrier films. In this study, commercially processed turkey slices with nitrate salt were stored under both atmospheric and saturated CO_2 atmosphere to simulate common industry and household practices. Turkey slices were stored at 4°C and 10°C for 70 days throughout the experiment. The log counts of *L. monocytogenes* on atmospheric-stored turkey slices decreased by 1.49 log CFU/g after 70 days at 4°C, but increased by 1.22 log CFU/g after 35 days at 10°C (Fig. 2.3B). The log counts of *L. monocytogenes* on MA-stored turkey slices stored decreased by 0.29 log CFU/g after 70 days at 4°C and by 1.21 log CFU/g after 70 days at 10°C (Fig. 2.3A). Fungal growth was observed on atmospheric-stored turkey slices after 21 days. No yeast or mold growth or off-odor was observed on MA-stored turkey slices. The data showed that abuse refrigeration temperature significantly hastened the growth of *L.*

monocytogenes on atmospheric-stored turkey slices after 49-70 days compared to strict refrigeration temperature ($p < 0.05$). However, no significant difference was observed between *L. monocytogenes* counts on MA-stored turkey slices under two refrigeration temperatures throughout 70 days of storage.

2.4.3 Effect of surface integrities on *L. monocytogenes* growth on whole produce

A three-way ANOVA test was performed to evaluate statistically significant differences between the average log counts of inoculated *L. monocytogenes* grown on either intact surfaces or cut wounds of fresh-cut lettuce leaves, tomatoes, and apples at 4°C and 10°C. No significant difference was observed between *L. monocytogenes* log increases on samples with intact surfaces and cut wounds two hours after inoculation. The log counts of inoculated *L. monocytogenes* on lettuce leaves with intact surfaces decreased over time, and was under the limit of detection at the end of the experiment at both 4°C and 10°C (Fig. 2.4A). Meanwhile *L. monocytogenes* inoculated on cut wounds increased by 1.05 log CFU/g after 23 days at 4°C and 1.38 log CFU/g after six days at 10°C (Fig. 2.4B). The results from the three-way ANOVA suggested that lettuce leaves with cut wounds were significantly more susceptible to bacterial growth after two days of storage ($p < 0.001$), and the differences remained significant throughout the entire experiment under abuse refrigeration temperature. When stored under strict refrigeration temperature, the significant differences between log growths on intact surfaces and cut wounds occurred after 16 days of storage and remained significant for another seven days.

Ripe-picked tomatoes are often stored at no lower than 10°C for 2-3 weeks to maintain quality. The log growths of inoculated *L. monocytogenes* on tomatoes with intact surfaces, damaged surfaces, and cuticle cracking, were measured and compared throughout 27 days of storage. No significant difference was observed among log counts on intact, damaged surfaces, and cuticle cracking, two hours after inoculation. Inoculated *L. monocytogenes* counts on tomatoes with cuticle cracking increased by 0.88 log CFU/g and 1.46 log CFU/g after 23 days of storage at 4°C and 10°C, respectively (Fig. 2.6C). Meanwhile the yeast and mold counts increased by 3.02 log

CFU/g at 4°C and 3.56 log CFU/g at 10°C after 23 days of storage. Whereas the log counts of yeast and mold on split tomatoes at both 4°C and 10°C exceeded 5 log CFU/g after two days of storage (Fig. 2.6C). The counts of inoculated *L. monocytogenes* on U.S. No.1 grade tomatoes and culled tomatoes with physiological defects were under the limit of detection at the end of the experiment at both temperatures tested (Fig. 2.6A&B). However, the growth rates of tomatoes with cuticle cracking were not significantly different from the ones with U.S. No.1 quality or physiological defects.

Tree-picked apples are often stored for 3-4 months with atmospheric storage and for almost a year with controlled atmosphere storage. The counts of *L. monocytogenes* inoculated on fresh-cut apple slices and tree-picked apples with U.S. No.1 quality and physiological defects were measured and compared throughout 27 days of storage. The *L. monocytogenes* counts were below limit of detection on U.S. No.1 grade apples after 2 hours of inoculation (Fig. 2.5A). Even though the average counts of inoculated *L. monocytogenes* on apples with physiological defects were significantly higher than those with U.S. No.1 quality two hours after inoculation ($p = 0.0029$), the counts decreased to below the limit of detection on apples with physiological defects after 48 hours of storage under both refrigeration temperatures (Fig. 2.5B). Yeast and mold counts on apples with physiological defects were 1.1 log CFU/g and 2.05 log CFU/g more than those on U.S. No.1 grade apples after 27 days of storage under strict and abuse refrigeration temperatures, but no quality deterioration appeared on either U.S. No.1 grade apples or culled apples with physiological defects throughout 27 days of storage under strict and abuse refrigeration temperatures. In contrast, the *L. monocytogenes* counts on fresh-cut apple slices, as mentioned previously, increased by 1.3 log CFU/g after six days under strict refrigeration temperature and 2.84 log CFU/g after four days under abuse refrigeration temperature (Fig. 2.5C).

The previous study on bacterial retention on secondary quality produce has shown that produce with intact surfaces retained significantly less inoculated *E. coli* than did that with physical damage (surfaces with broken cuticles) following

disinfection treatment (Cai et al., unpublished). Subsequently, the data from this study suggests that the rapid growth of spoilage microbiota limits the shelf life of outgraded produce with physical damage, and thus makes the survival and growth of retained pathogens, during post-harvest storage, less relevant to food safety.

Table. 2.1: Summary of *Listeria monocytogenes* and spoilage microbiota growth rate on various RTE foods

		4°C			10°C		
Food type	**Shelf life**	LM growth	Spoilage growth	Sign of Spoilage (Yes/No)	LM growth	Spoilage growth	Sign of Spoilage (Yes/No)
Fresh-cut cantaloupe slices under atmospheric storage	3 days	0.9 log CFU/g after 2 days	1.18 log CFU/g after 2 days	No	1.05 log CFU/g after 1 day	0.48 log CFU/g after 1 day	Yes after 3 days
Spinach leaves under atmospheric storage	16 days	1.43 log CFU/g after 20 days	2.63 log CFU/g after 20 days	No	0.98 log CFU/g after 2 days	1.57 log CFU/g after 2 days	Yes after 13 days
Pre-cut lettuce leaves under atmospheric storage	16 days	0.06 log CFU/g after 27 days	2.21 log CFU/g after 27 days	Yes after 27 days	0.98 log CFU/g after 6 days	2.28 log CFU/g after 6 days	Yes after 13 days
Pre-cut lettuce leaves with modified atmosphere storage	16 days	1.14 log CFU/g after 20 days	2.09 log CFU/g after 20 days	No	0.98 log CFU/g after 2 days	0.92 log CFU/g after 2 days	Yes after 27 days
Turkey slices with atmospheric storage	70 days	-1.49 log CFU/g after 70 days	Under detection limit after 70 days	No	1.22 log CFU/g after 35 days	0.26 log CFU/g after 35 days	Yes after 21 days
Turkey slices with modified atomosphere storage	70 days	-0.29 log CFU/g after 70 days	0.44 log CFU/g after 70 days	No	-1.21 log CFU/g after 70 days	Under detection limit after 70 days	No
Pre-cut lettuce leaves with intact surface inoculation	16 days	Under detection limit after 23 days	-0.74 log CFU/g after 23 days	No	Under detection limit after 23 days	-0.64 log CFU/g after 23 days	No
Pre-cut lettuce leaves with cut wound inoculation	16 days	1.05 log CFU/g after 23 days	1.41 log CFU/g after 23 days	Yes after 23 days	1.38 log CFU/g after 6 days	0.83 log CFU/g after 6 days	Yes after 6 days
Ripe-picked tomatoes with cuticle cracking	21 days	0.88 log CFU/g after 23 days	3.02 log CFU/g after 23 days	Yes after 2 days	1.46 log CFU/g after 23 days	3.56 log CFU/g after 23 days	Yes after 2 days
Ripe-picked tomatoes with intact surfaces	21 days	Under detection limit after 6 days	0.57 log CFU/g after 27 days	No	Under detection limit after 2 days	0.3 log CFU/g after 27 days	No
Ripe-picked tomatoes with damaged surfaces	21 days	Under detection limit after 13 days	-0.87 log CFU/g after 27 days	No	Under detection limit after 6 days	1.3 log CFU/g after 27 days	Yes after 9 days
Fresh-cut apple slices with air headspace	10 days	1.3 log CFU/g after 6 days	2.24 log CFU/g after 6 days	Yes after 12 days	2.84 log CFU/g after 4 days	4.55 log CFU/g after 4 days	Yes after 6 days
Tree-picked apples with intact surfaces	4 months	Under detection limit after 2 hours	0.2 log CFU/g after 27 days	No	Under detection limit after 2 hours	Under detection limit after 27 days	No
Tree-picked apples with damaged surfaces	4 months	Under detection limit after 2 days	1.3 log CFU/g after 27 days	No	Under detection limit after 2 days	0.68 log CFU/g after 27 days	No

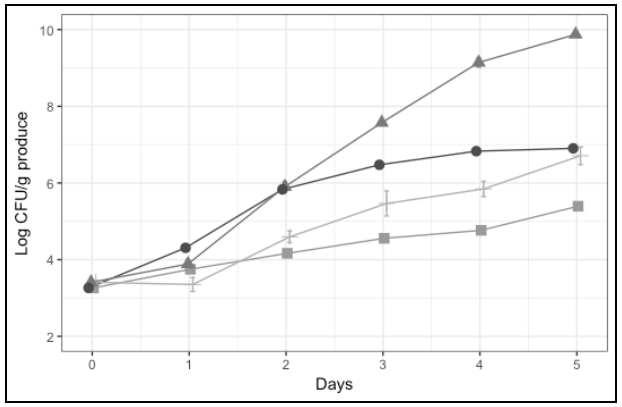

Fig. 2.1: Growth curve of *L. monocytogenes* at 4°C (■), *L. monocytogenes* at 10°C (●), yeast at 4°C (+), yeast at 10°C (▲) on fresh-cut cantaloupe under atmospheric storage.

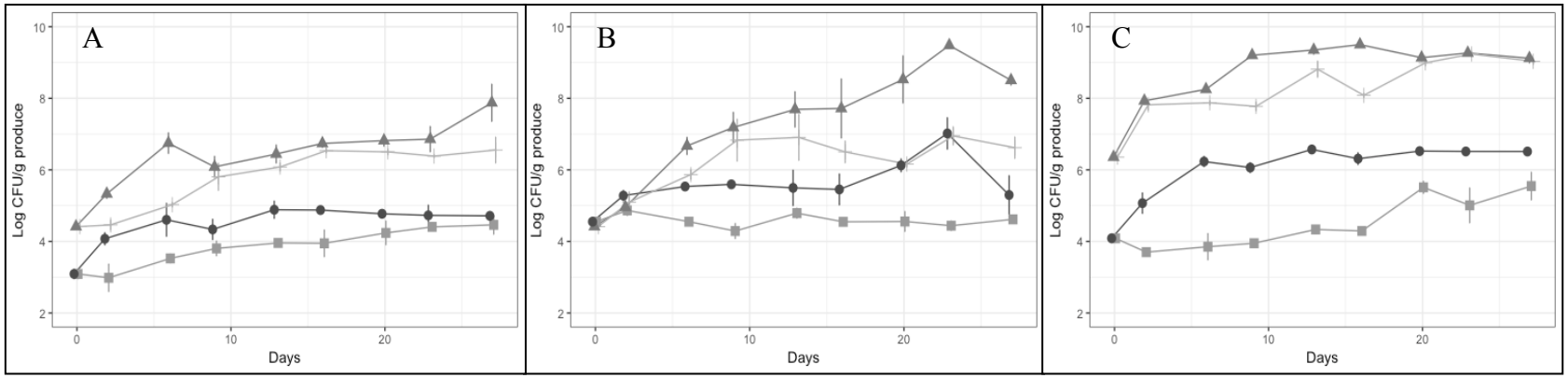

Fig. 2.2: Growth curve of *L. monocytogenes* at 4°C (■), *L. monocytogenes* at 10°C (●), Gram-negative bacteria at 4°C (+), Gram-negative bacteria at 10°C (▲) on: (A) fresh-cut lettuce leaves under modified atmosphere storage, (B) fresh-cut lettuce leaves under atmospheric storage, and (C) baby spinach leaves under atmospheric storage.

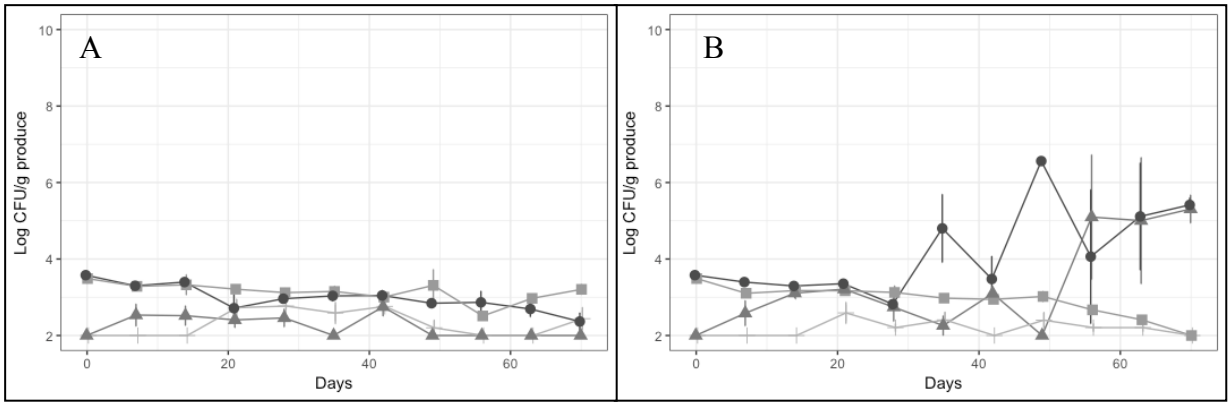

Fig. 2.3: Growth curve of *L. monocytogenes* at 4°C (■), *L. monocytogenes* at 10°C (●), lactic acid bacteria at 4°C (+), lactic acid bacteria at 10°C (▲) on commercially processed turkey slices under: (A) modified atmosphere storage, and (B) atmospheric storage.

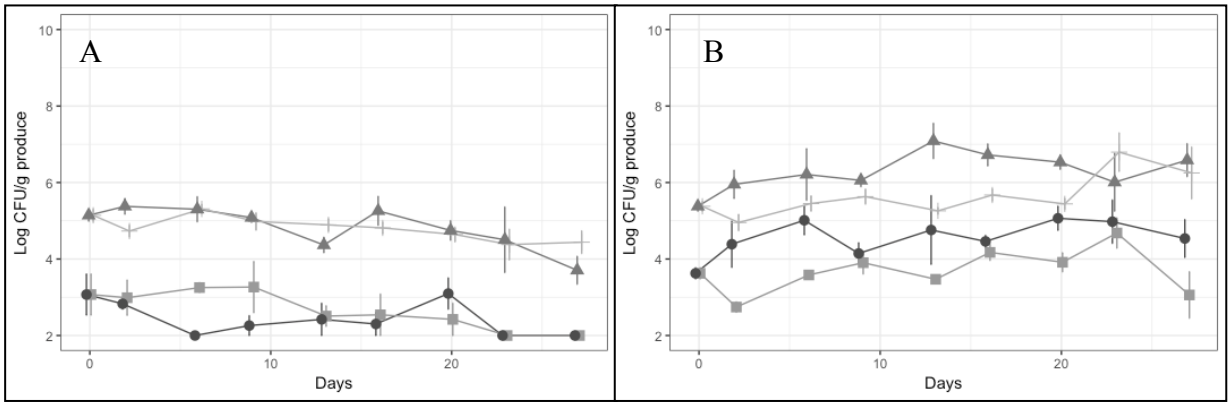

Fig. 2.4: Growth curve of *L. monocytogenes* at 4°C (■), *L. monocytogenes* at 10°C (●), Gram-negative bacteria at 4°C (+), Gram-negative bacteria at 10°C (▲) on lettuce leaves with: (A) intact surfaces and (B) cut wounds.

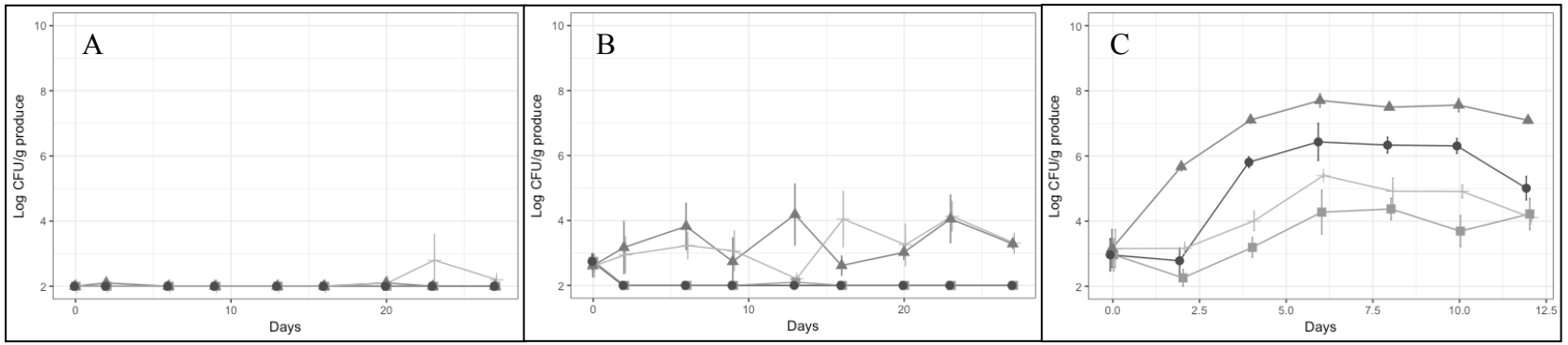

Fig. 2.5: Growth curve of *L. monocytogenes* at 4°C (■), *L. monocytogenes* at 10°C (●), yeast and mold at 4°C (+), yeast and mold at 10°C (▲) on: (A) U.S. No. 1 quality tree-picked apples, (B) tree-picked apples with physiological defects, and (C) fresh-cut apple slices.

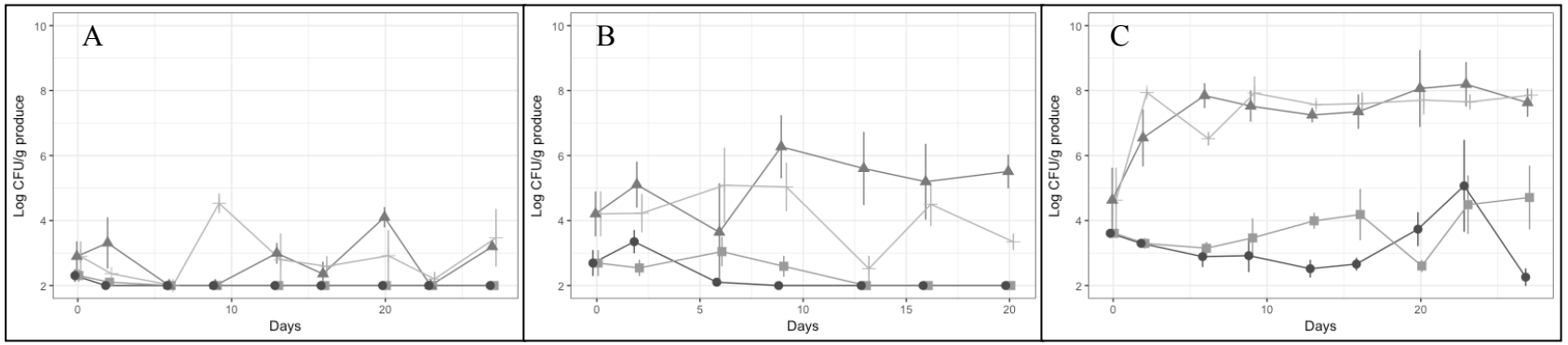

Fig. 2.6: Growth curve of *L. monocytogenes* at 4°C (■), *L. monocytogenes* at 10°C (●), yeast and mold at 4°C (+), yeast and mold at 10°C (▲) on: (A) U.S. No. 1 quality ripe-picked tomatoes, (B) ripe-picked tomatoes with physiological defects, and (C) tomatoes with cuticle cracking.

2.5 Discussion

The combined impact of storage temperatures and length of the storage period on inoculated *L. monocytogenes* and native spoilage microbiota growth under refrigerated storage was evaluated on six model RTE product systems (tomatoes, apples, fresh-cut cantaloupe slices, fresh-cut lettuce leaves, baby spinach leaves, and commercially processed turkey slices). Additionally, the integrity of the surface structure was also evaluated as a mediating parameter. The factors affecting the growth of *L. monocytogenes* and interactions between *L. monocytogenes* and spoilage microbiota in food are shown in Fig. 2.1-2.6. Although FDA has a zero tolerance for *L. monocytogenes*, the 2013 FDA Food Code permits environmental conditions that allow *L. monocytogenes* cells 1 log of growth in RTE food (USPHS, 2013). This study applied the metric of a 10-fold growth mark of *L. monocytogenes* growth as an indication of largely increased risk to human health, based on the critical limit set by the FDA Food Code. Generally, when any of the hurdles to bacterial growth are disrupted, the counts of *L. monocytogenes* increased by 10-fold before the quality of food deteriorated under both strict and abuse refrigeration temperatures.

Plant epidermic tissues serve as an intrinsic barrier affecting the growth of *L. monocytogenes*. Most unprocessed food derived from plant or animals have a natural protective physical barrier that hinders microorganisms from entry into the cells and tissues of the food (Hamad, 2012). It has also been reported that injured or fresh-cut produce have a higher final population of *L. monocytogenes* than whole produce after storage, and elevated temperature hasten microbial growth on injured surfaces (Han et al., 2001; Beuchat & Scouten, 2004; Brandl 2008). Physical damage from insect attacks, or processing practices, such as slicing, disrupts the intact skin and releases cellular liquid containing nutrients for the microorganisms. Subsequently, food without its intact skin as a protective barrier would bear an increased risk from *L. monocytogenes* growth. *L. monocytogenes* and spoilage microbiota counts on U.S. No.1 quality apples and tomatoes were below limit of detection at the end of the storage period. The counts of *L. monocytogenes* on culls with physiological defects also decreased to below the limit of detection, however, the spoilage microbiota

counts were significantly greater than the ones of U.S. No.1 quality apples at the end of the storage. Meanwhile, *L. monocytogenes* on fresh-cut cantaloupe, fresh-cut apple, baby spinach leaves, and lettuce leaves with cut wounds, increased by more than 1 log CFU/g before or on the same day when the signs of spoilage were evident. Even though the quality of ripe-picked tomatoes with cuticle cracking deteriorated at the early stage of storage before *L. monocytogenes* reached a 1 log CFU/g increase, little to no signs of spoilage were observed on the rest of the samples with open lesions at the end of the storage period when stored under strict refrigeration temperature (Table 2.1). This suggests that strategies used for quality control cannot be relied upon for controlling the growth of *L. monocytogenes* when the product has been cut or injured in some way.

Innovative packaging technologies, such as modified atmosphere packaging, can also be one of the factors affecting the growth of *L. monocytogenes*, although they are primarily designed with preservation of biotic and abiotic quality as targets. It has long been known that the combination of chilling and control/modification of the gas atmosphere can greatly enhance the preservative effect during storage (Robertson, 2005). Fang & Lin (1994) reported that saturated CO_2 atmosphere largely inhibited the growth of *L. monocytogenes* on cooked pork at 4°C compared to 20°C (Table. A2.1). A previous study showed evidence that modified atmosphere did not exert a bactericidal effect on RTE shrimp under abuse refrigeration temperatures (Rutherford et al., 2007). In this study, *L. monocytogenes* counts on turkey slices decreased over time during the storage before quality deterioration appeared, likely due to the presence of preservatives (nitrite and salt). Even though *L. monocytogenes* on atmospheric-stored turkey slices under abuse refrigeration temperature increased by more than 1 log CFU/g during shelf-life storage, fungal growth appeared two weeks early. The quality control of commercially processed turkey slices with nitrite salts appeared to effectively inhibit the growth of *L. monocytogenes*. In addition, few studies on the behavior of *L. monocytogenes* under modified temperatures at strict and abuse refrigeration temperatures were found relating to fresh-cut fruits and vegetables and the interface between safety and quality (Table A2.1). Conway et al. (2000)

reported that the effect of modified atmosphere on untreated fresh-cut apples slices was weakened under abuse refrigeration temperature. The data in this study showed that modified atmosphere storage largely preserved the quality of fresh-cut lettuce leaves under both refrigeration temperatures, whereas did not exert any bactericidal effect on *L. monocytogenes*. Said data suggests that using quality attributes to determine the shelf life of select RTE products may increase food safety risks on products that support the growth of *L. monocytogenes*. Risk-based guidance should also consider the incidence of contamination, frequency with which products are temperature and shelf life abused, and trade-offs concerning food waste.

In conclusion, the quality deteriorations should not be used as fail-safe indicators considering shelf-life limitation for *L. monocytogenes* growth on fresh-cut products. The growth of *L. monocytogenes* becomes problematic prior to consumer sensory deterioration thresholds with foods under refrigerated storage. Whereas, rapid growth of spoilage microbiota limits the shelf life of outgraded produce with physical damage, and thus makes the survival and growth of retained pathogens, during post-harvest storage, less relevant to food safety. Therefore, select U.S. No. 1 graded fruit (tomatoes, apples), along with the culls, with or without open lesions, pose less food safety risks from *L. monocytogenes* growth under refrigerated storage compared to the fresh-cut products.

Conflict of interest

The authors declare no conflict of interest.

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APPENDIX B

Table. A2.1: Summary of previously reported *Listeria monocytogenes* growth rate on RTE food

Food Category Reference	**Food**	**Literature Values**	
		Temperature (°C)	Growth Rate
Danyluk et al., 2014	Fresh-cut cantaloupe	4	1-log CFU/g in 6 days
		5	4-log CFU/g in 15 days
Leverentz et al., 2003	Fresh-cut honeydew melon slices	10	4.46-log CFU/g in 7 days
	Fresh-cut apple slices	10	0.56-log CFU/g in 7 days
Vandamm et al., 2013	Fresh cut celery	4	decrease
		12	0.5-log CFU/g in 7 days
		22	0.3-log CFU/g in 7 days
Conway et al., 2000	Fresh-cut apple slices with air storage	5	0-log CFU/g in 6 days
		10	2-log CFU/g in 6 days
	Fresh-cut apple slices with modified atmosphere storage	5	0-log CFU/g in 4 days
		10	2.8-log CFU/g in 10 days
Salazar et al., 2016	Gala caramel apples	25	1.64-log CFU/g per day
	Fresh gala apples		no growth in 49 days
	Granny Smith caramel apples		1.38-log CFU/g per day
	Fresh Granny Smith apples		no growth in 49 days
Sheng et al., 2017	Whole Granny Smith apples	1,4,10	0.2-0.3-log CFU/g decrease in 14 days
		22	0.5-1.2-log CFU/g decrease in 14 days
		1,4,10	0.5-1.5-log CFU/g decrease in 12 weeks
		22	0.8-2-log CFU/g decrease in 3 months
Han et al., 2001	Green peppers with intact surface	7	0.63-log CFU/g in 14 days
	Green peppers with artificially injured surface	7	1.42-log CFU/g in 14 days
Ells & Hansen, 2010	Cut cabbage	5	1.2-log CFU/g in 14 days
Steinbrugge et al., 1988	Whole lettuce, ready to serve	5	0.00-0.3-log CFU/g in 7 days
		12	0.00-2.03-log CFU/g in 7 days
	Whole lettuce, ready to serve, sealed	25	0.00-0.31-log CFU/g in 7 days
	Whole lettuce, ready to serve, open	25	0.00-0.35-log CFU/g in 7 days
Beuchat and Brackett, 1990a	Shredded lettuce	5	0.00-0.1-log CFU/g in 15 days
	Shredded lettuce	10	1.5-2.0-log CFU/g in 3 days
	Whole lettuce	10	1.0-log CFU/g in 5 days

Table. A2.1: Summary of previously reported *Listeria monocytogenes* growth rate on RTE food (Continued)

Food Category Reference	Food	Literature Values	
		Temperature (°C)	Growth Rate
Nguyen and Carlin,1994	Butterhead lettuce	10	1.5-log CFU/g in 7 days
	Lamb's lettuce	10	1.0-log CFU/g decrease in 7 days
Carlin et al., 1995	Broad leaved endive	10	1.0-log CFU/g in 7 days
Nguyen and Carlin,1994	Broad leaved endive	10	1.5-log CFU/g in 7 days
	Curly leaved endive	10	0.5-log CFU/g in 7 days
Beuchat and Brackett,1991	Whole tomatoes	10	no growth
		21	no growth
Glass and Doyle, 1989	Cooked ham	4.4	2-3-log CFU/g in 28 days
Glass and Doyle, 1989	Bologna	4.4	1-2-log CFU/g in 14 days
	Vacuum packed chicken slices		4.15-log CFU/g in 14 days
			5.90-log CFU/g in 14 days
	Turkey slices	4.4	2-log CFU/g in 14 days
			3.11-log CFU/g in 28 days
			3.08-log CFU/g in 14 days
	Vacuum-packed turkey slices		3.83-log CFU/g in 14 days
			5.09-log CFU/g in 14 days
Zhu et al., 2009	Turkey breast rolls	4	2.2-log CFU/g in 14 days
Lianou et al., 2006	Commercial ham without antimicrobials	7	0.32-0.45-log CFU/g in 4 days
	Commercial ham with antimicrobials	7	0.18-0.25-log CFU/g in 8 days
Fang and Lin, 1994	Cooked pork with air storage	4	2.78-log CFU/g in 6 days
		20	4.20-log CFU/g in 1 day
	Cooked pork with saturated CO2 atmosphere storage	4	2.16-log CFU/g in 12 days
		20	1.90-log CFU/g in 1 day

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